FISH FROM GREECE

Mediterranean Fish Welfare:
Guide to good practices and assessment indicators

HAPO
HELLENIC AQUACULTURE PRODUCERS ORGANIZATION

December 2019

The content of this edition of the «Mediterranean Fish Welfare: Guide to Good Practices and Assessment Indicators» has been approved by the Directorate of Animal Protection and Veterinary Drugs in the Ministry of Rural Development and Food.

Suggested citation:

M. Pavlidis & A. Samaras (2020). Mediterranean Fish Welfare: Guide to Good Practices and Assessment Indicators, pp 90

Members of HAPO[1] and UoC[2] involved (listed alphabetically)
A. Frentzos[1], M. Kolygas[1], L, Papaharisis[1], I. Tsikopoulou[2]

Administrative support: S. Kyriakidi[2]

Finance by the Hellenic Aquaculture Producers Association (HAPO)

Professor Michail Pavlidis
Department of Biology,
University of Crete

Dr. Athanasios Samaras
Postdoctoral Researcher,
University of Crete

Working Group (in alphabetical order)

Athanasios Frentzos,
Ichthyologist, MSc

Dr. Markos Kolygas,
Veterinarian, Aquatic Pathobiologist, DVM, BSc-Tech, MSc, Post-Doc

Leonidas Papaharisis,
Ichthyologist, MPhil

Professor Michail Pavilidis,
Department of Biology, University of Crete

Dr. Athanasios Samaras,
Postdoctoral Researcher, University of Crete

Dr. Irini Tsikopoulou,
Postdoctoral Researcher, University of Crete

Acknowledgements

Text edited by Ms. Klarita Tusku; all photographs were kindly provided by Ms. Yukiko Krontira at Kefalonia Fisheries S.A. Translation from Greek by Mr. Ben Petre.

Contents

Introduction

Fish are the most numerous and varied group of vertebrates. Over 33,000 species are found in all aquatic ecosystems, from the extreme environments at the poles to warm tropical waters, from fresh water to isolated, highly saline areas and from shallow bodies of water to the great depths of the oceans. Fish display remarkable morphological, physiological and behavioural adaptations in securing their survival and establishing a firm foothold in a constantly changing, three-dimensional natural world. There are species living in environments with minor fluctuations in salinity (stenhohalines) and/or temperature (stenotherms), others that can thrive in wider ranges (euryhalines and eurytherms), species that have high oxygen demands (e.g. trout and salmon), and others adapted to survive in oxygen-poor waters (e.g. catfish and cyprinids). Lastly, there are species that live alone, and others that form shoals consisting of large numbers of individuals.

For billions of humans, fish are an important nutrient-rich source of food, which is provided both by fishing and by aquaculture. The contribution of fisheries to world global fish production has remained stagnant since the late 1980s, whereas aquaculture has trended upwards, reaching 47% of overall output in 2016 (http://www.fao.org/state-of-fisheries-aquaculture). In Europe, aquaculture accounts for 20% of total fish output and is a major economic activity, employing approximately 85,000 people (http://feap.info/). In Greece, the farming of Mediterranean sea fish has been one of the fastest growing animal production sectors for the past three decades, with prospects for further growth. According to a recent report by the Federation of Greek Mariculture, Greek output already accounts for 61% of sea bass and sea bream production in the EU, and almost 30% of global production, with distribution to over 32 countries (FGM, 2018). 62% of total domestic fish product output comes from mariculture, an important branch of the primary production sector that has created 12,000 direct and indirect jobs, mainly in coastal or remote areas (FGM, 2018, van de Vis *et al*., 2017).

In contrast to other production animals, it is only relatively recently that farmed species of fish and welfare issues relating to them have aroused scientific concern and public interest (Huntingford *et al*., 2006). Yet fish welfare is now a major priority just as much for producers and all professionals involved in the fish farming sector as for the scientific community, consumers, NGOs, regulatory bodies and the state.

Producers have an especial interest in the welfare of the animals they farm. They have developed and adopted practices and technologies leading to the assessment of fish condition, performance monitoring and welfare improvement. On the other hand, management and regulatory bodies as well as independent organisations have developed standards and certified welfare schemes for particular species of farmed fish. This situation has set the sector new challenges, including that of obtaining proper information on current scientific views and the relevant legal framework.

Within that context, aquaculturists wish to incorporate acceptable and reliable welfare indicators into the production process. In taking daily care of their stock, they wish to incorporate husbandry practices that improve fish welfare and vigour. Yet this in itself represents a challenge, given that the concept of welfare is complex: it has an objective dimension to the individual experiencing it, and is liable to modification on the basis of current scientific and philosophical views. Objectively "measuring" also represents a challenge, as available welfare assessment indicators may not be suitable for all farmed species of fish and/or may not be implementable at all life cycle phases and in all production systems. In the case of fish, there is the added difficulty of monitoring and recording the state of separate individuals in a large shoal moving in three-dimensional space. Lastly, one further difficulty is the lack of reference values for blood, biochemical and hormonal laboratory welfare indicators.

In assessing welfare, specific, measurable Welfare Indicators (WIs) have been developed. These can be subdivided into direct indicators (relating to fish) and indirect ones (relating to the environment or farming and operations technology). They are also separated into Operational Welfare Indicators (OWIs), i.e. those implementable on the farm and in the various phases of the production process, and Laboratory-based Welfare Indicators (LBWIs), based on samples taken from fish on the farm and sent to specialist laboratories for analysis. Lastly, there are indicators now being developed at the research level, which may be used in the production process at some point in the future.

Aim and scope of the guide

The main aim is to compile an operational guide for workers and those involved in mariculture, so that they can carry out tasks in line with current scientific knowledge on the welfare of farmed fish and well-established farming knowhow.

As regards implementation, this guide is for the production cycle of European sea bass and gilthead sea bream, i.e. the two main species in Mediterranean Aquaculture. For each of the individual production stages and each main activity, practices limiting fish stress and improving welfare will be described. The guide will set out the optimal practices for reducing stress and name the Operational Welfare Indicators, linking them to the tasks carries out at production facilities. In addition, measurable Laboratory Welfare Indicators will be given, confirming the impact of the sound practices adopted. The individual production stages and activities to which the guide makes reference are:

Phases	Processes
❖ Breed Management	❖ Sorting
❖ Pre-growing	❖ Sampling to monitor weight and health
❖ Juvenile Transport	❖ Administering Veterinary Treatments
❖ On-growing	❖ Diving Work
❖ Harvesting	❖ Feeding

The main scope of the guide is:

1. To briefly describe current scientific data and ethical views on animal welfare.

2. To describe the needs of European sea bass and gilthead sea bream in different production phases, in relation to improving their welfare.

3. To describe the Operational and Laboratory Welfare Indicators, evaluating the advantages and disadvantages of each indicator.

4. To assess the welfare indicators in relation to production system, production phase and husbandry practices employed.

5. To reinforce practices leading to the improvement of fish welfare.

6. To produce material to further train employees in the mariculture sector on welfare issues.

This guide is based on study of relevant scientific literature, publications by international organisations and guides published on other farmed fish species (Noble *et al*., 2018). It represents the first such attempt in Greece, aiming to serve as an open feedback process for its further improvement. This is a process that may result in the development of welfare indicators for European sea bass and gilthead sea bream that are acceptable to producers as well as to regulatory bodies and consumers.

1 | 1. Theoretical framework and scientific views on welfare

At least in the so-called Western world, social sensitivity towards the correct treatment and welfare of farmed mammals and birds is taken for granted. On the other hand, producers are well aware that rearing conditions have a decisive impact on the quality of meat produced. Within that context, sound practice rules and regulatory frameworks have been developed with regard to the best possible care of terrestrial farm animals during rearing and transport to abattoirs. Lastly, recommended humane killing techniques have been developed.

In contrast to other production animals, it is only relatively recently that farmed species of fish and welfare issues relating to them have aroused scientific concern and public interest (Huntingford *et al*., 2006). Yet fish welfare is now a major priority just as much for producers and all professionals involved in the aquaculture sector as for the scientific community, consumers, NGOs, regulatory bodies and the state.

Views on welfare differ, often leading to heated debates in the public arena. This is easy to see once we grasp that the concept of welfare has philosophical, scientific and legal dimensions, which are referred to in brief in the following section.

1.1 | Philosophical views on welfare

The two fundamental philosophical schools dominating the debate on welfare are utilitarianism (consequentialism) and deontology (Rachels and Rachels, 2010). Utilitarians argue that humans should do what leads to the greatest possible happiness for the greatest number of individuals, given that humans are beings capable of feeling pain, pleasure and joy. Utilitarianism places emphasis on the consequences or results of our actions. A correct action is one tending to maximise benefit. According to Peter Singer (1975), the leading proponent of the utilitarian tradition in the modern era, animals capable of sentience are to be included in the moral community of humans. On the other hand, the school of deontology lays down moral values up front, placing the morality of an action in the action itself. This school accords rights to those beings that have inherent value. According to Tom Regan (1983), a contemporary proponent of the deontological tradition, animals of inherent value are those possessing self-consciousness, beliefs, desires, goals and awareness of the future.

That being said, regardless of moral theories and their philosophical roots, the main issue is whether "*humans acknowledge moral obligations towards animals, and whether they are willing to fulfil them*" (Haziroglou, 2018). Yet how each human being understands the concept of welfare in their everyday life has to do with their personal values, their personal interests and the society they live in. In general terms, views head in three directions (Fraser, 2008; Vapnek and Chapman, 2011). The first (functional-physiological) approach sees welfare as synonymous with an animal's good health (lack of injuries, abrasions and diseases) and the proper functioning of its natural mechanisms. The second (affective) approach places emphasis on the animal's emotions and the avoidance of negative situations such as pain, fear, stress, anxiety and hunger. The third (natural living) approach mainly refers to an animal's need to exhibit its innate-natural behaviour as expressed in its natural habitat (this view is particularly widespread in countries where the super-intensive rearing of production animals predominates).

1.2 | Scientific research and welfare

Research into the welfare of animals used for scientific and production purposes is cross-disciplinary, including a range of fields, such as genetics, molecular biology and high-performance biomolecular analysis (omic technologies), physiology and the neuroendocrinology of stress, nutrition, ethology, pathology, and disease prevention and treatment. It aims at gaining better knowledge of animal function, needs and desires, developing reliable welfare indicators and improving rearing conditions, and strives to answer questions relating to pain, anxiety, fear, sentience, empathy and consciousness. At the same time, it includes all stages in an organism's life cycle and different production phases, in relation to technology and rearing system. Historically speaking, such research has for the most part concerned mammals and birds, extending to fish relatively recently.

The truth is that to this day, no commonly accepted definition of animal welfare has been arrived at. It is also worth stressing that there is no commonly accepted translation of the term into the Greek language. It is often used as synonymous with fair treatment, the absence of stress, fear and anxiety, good health, wellbeing and good quality of life. In this guide we shall use the term as rendered in Greek legislation, as defined by Broom (1986), and adopted in its expanded version by the World Organisation for Animal Health (OIE, 2008):

"*Welfare is the state of an animal in relation to its ability to cope with its environment. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well-nourished, safe, able to express innate behavior, and is not suffering from unpleasant states such as pain, fear or distress.*"

Achieving welfare means providing suitable rearing conditions, implementing suitable steps to prevent diseases and provide effective veterinary treatment of them, proper husbandry practices and operations, as well as employing a humane killing method.

1.3 | Regulatory framework

The first law concerning the prevention of cruelty to production animals ("An Act to prevent the cruel and improper Treatment of Cattle") was passed by the parliament of the United Kingdom in 1822. It is otherwise known as Martin's Law, after the Irish parliamentarian who introduced the law and also founded the first animal welfare organisation, the Royal Society for the Prevention of Cruelty to Animals (RSPCA) in 1824 (https://en.wikipedia.org/wiki/Cruel_Treatment_of_ Cattle_ Act_1822). Laws in other European countries then followed. 1978 saw the signing in Paris of the Universal Declaration of Animal Rights, a symbolic text which proclaims, among other things, that: "*Where animals are used in the food industry, they must be fed, managed, transported and killed without them being in fear or pain.*" (Article 9).

In 2001, Presidential Decree 374 on "The protection of animals in farming facilities" was published in Greece, in compliance with European Directive 98/58/EC implementing Council Decision 2000/50/ EC, "Concerning the protection of animals kept for farming purposes" (A 251). This decree also applies to farmed fish species (Article 2), though special provisions on rearing conditions and processes are only given for calves, pigs and laying hens (Article 4 and Annex).

The Treaty of Lisbon, signed in 2007, recognises animals used for scientific and production purposes as "sentient beings", and calls upon Member States to adapt their legislation in accordance with what is laid down in article 13 ("In formulating and implementing the Union's agriculture, fisheries, transport, internal market, research and technological development and space policies, the Union and the Member States shall, since animals are sentient beings, pay full regard to the welfare requirements of animals, while respecting the legislative or administrative provisions and customs of the EU countries relating in particular to religious rites, cultural traditions and regional heritage.") *(https://ec.europa.eu/food/animals/welfare_en).*

2005 saw the publication of European Council Regulation (EC) 1/2005 "on the protection of animals during transport", followed in 2009 by Council Regulation (EC) No 1099/2009 on the "protection of animals at time of killing". Although these regulations also concern farmed species of fish, the first makes no mention of specific guidelines for them. In the second it is stated that: "*as regards fish, only the requirements laid down in Article 3(1) shall apply*", in other words, the general wording that: "*Animals shall be spared any avoidable pain, distress or suffering during their killing and related operations*", while in article 27 it is also affirmed that: "Pending the adoption of these rules [regarding the protection of fish when being slaughtered], *Member States may maintain or adopt national rules regarding the protection of fish at the time of slaughter or killing and shall inform the Commission thereof*". In 2017, a study was published at the behest of the European Commission on the welfare of farmed fish in regard to common practices during transport and harvesting. The report concerned five commercial species (salmon, trout, common carp, European sea bass and gilthead sea bream), but implementation of it was not legally binding (van de Vis *et al.*, 2017).

2013 saw the publication of Presidential Decree No 56, adapting Greek legislation to Directive 2010/93/EU of the European Parliament "on the protection of animals used for scientific purposes" (A' 106). For the first ever time, the decree makes additional mention of the zebrafish *(Danio rerio)*, a model organism in biomedical research. Commercial fish species are also included when used as animals for scientific purposes. The decree is not applicable to practices followed for recognised animal production purposes.

1.4 | Cognitive functions in fish

Fish possess the anatomical structures, bodily systems and cognitive functions to perceive individuals of the same species, other species and the environment in which they live. They have central and autonomic nervous systems, well-developed endocrine and sensory systems, as well as the ability to learn, remember, encode space, recognise individuals of the same species, develop complex social interactions and use tools (Brown, 2015). In addition to perceiving images, colours, sounds, tastes and smells, several species of fish are capable of perceiving the source of vibrations (via their lateral line and otoliths) and magnetic or electric fields. There are also species that can make sounds, and others that have light-producing or bioelectric organs.

Fishes are capable of reacting to adverse stimuli via a mechanism similar to that of mammals and humans. In stress situations, the teleost brain produces endocrine factors that act on chromaffin and interrenal tissue (structures corresponding to the medulla and adrenal cortex in mammals) to produce catecholamines (noradrenaline and adrenaline) and cortisol. This is the so-called primary "fight or flight" stress response, which goes on to trigger further metabolic, immune and behavioural responses. It is worth noting that this ability emerges during the first developmental stages (mouth opening) in gilthead sea bream and European sea bass (Szisch *et al.*, 2005; Tsalafouta *et al.*, 2015a, 2015b, 2018). It is now accepted that fish express genomic, physiological, neuroendocrine and behavioural responses similar to those expressed by mammals in situations of stress, fear and anxiety (Wendelaar Bonga, 1997; Tort *et al.*, 2011; Stewart *et al.*, 2012; Tort *et al.*, 2014; Balasch and Tort, 2019). Increase in respiration rate (opercular beat rate), "freezing behaviour", spending extended time on the bottom of tanks, preference for dark areas and loss of schooling consistency are all cited as characteristic examples of such behavioural responses (Maximino *et al.*, 2010; Martins *et al.*, 2012; Stewart *et al.*, 2012; Millot *et al.*, 2014; Kleinhappel *et al.*, 2019).

As far as pain sensitivity is concerned, most researchers consider that teleost fishes can feel pain. They possess nociceptors activated by painful mechanical, thermal or chemical stimuli, appropriate neural sensory fibres (mainly of the Aδ type, which relay information on pain at higher speed) to relay neural stimulation; neurotransmitters and metabolites (which have an important role to play in perceiving pain, vigilance, avoidance, reinforcement and reward); and they react to analgesic administration (Sneddon *et al.*, 2003; Ashley *et al.*, 2007; Braithwaite, 2010; Sneddon, 2019). Nevertheless, there are scientists who dispute the ability of fish to feel pain (Rose, 2002; Rose *et al.*, 2014; Key, 2016). Their arguments are mainly based on the limited presence (or total absence in several fish species) of group C nerve fibres (low conduction fibres that relay pain and serve tactile stimuli, such as light touch and pleasant touch), as well as the absence of the neocortex, i.e. the area of mammal brains that is the seat of higher cognitive functions including pain processing. Yet regardless of whether teleosts can feel pain in the same way as humans do, EU legislative authorities consider fish to be sentient animals, hence all necessary steps must be taken to eliminate or minimise any potential pain, distress or permanent damage inflicted by humans.

As mentioned above, fishes possess the ability to evaluate situations, learn and remember, and adapt and alter their behaviour depending on their genetic predisposition and the way they perceive their environment. They have a sense of space and time, show anticipatory behaviour, can recognise individuals of the same and other species, cooperate in a school and manifest social interactions (Anthouard, 1987; Gómez-Laplaza and Gerlai, 2011; Millot *et al.*, 2014; Vail *et al.*, 2014; Brown, 2015). That being said, the question of whether they have consciousness remains open to question. This is only reasonable considering that there are different approaches to the definition of consciousness, and scarcely any data on how higher conscious abilities developed in humans.

1.5 | Welfare Indicators

In assessing the welfare of fish, Welfare Indicators can be employed. These indicators can be subdivided into:

(i) Indirect or environmental indicators, which relate to environmental characteristics such as oxygen saturation or water salinity, and
(ii) Biological indicators, where the information concerns the fish itself, such as health, growth and appetite.

Beyond this distinction, indicators are also separated into:

(a) Operational indicators, i.e. those which can be assessed in situ, on the fish farm, and

(b) Laboratory-based indicators, which require specially trained staff to take samples for analysis by a remote specialist laboratory.

Table 1.1. Classifying welfare indicators. Typical examples are given for each category.

Welfare Indicators			
Indirect	Biological	Operational	Lab-based
- Oxygen - Salinity - Stocking density	- Health - Nutrition - Behaviour	- Oxygen - Nutrition - Growth	- Health - Cortisol - Maturation

1.6 | Summary

•Fish are the most numerous and varied group of vertebrates. Over 33,000 species are found in all aquatic ecosystems, from the extreme environments at the poles to warm tropical waters, from fresh water to isolated, highly saline areas and from shallow bodies of water to the great depths of the oceans.

•They have central and autonomic nervous systems, well-developed endocrine and sensory systems, as well as the ability to learn, remember, encode space, recognise individuals of the same or other species, cooperate in a school, and manifest complex social interactions.

•Regardless of their degree of consciousness or sense of pain, fish are sentient animals, and consequently all necessary steps must be taken to eliminate or minimise any potential pain, distress or permanent damage that may be inflicted on them by humans.

2 | Biological needs and welfare

2.1 | Rearing water quality

European sea bass and gilthead sea bream are eurythermal and euryhaline species, and also show tolerance to a wide range of dissolved oxygen saturation. Temperature tolerance/survival range is 2-35°C for European sea bass (EFSA, 2008; Dülger *et al*., 2012) and 5-34°C for gilthead sea bream (EFSA, 2008). Since tolerance depends on acclimatisation period, gradual alterations in temperature are not regarded as stressful, whereas particularly near tolerance limits, sudden changes may negatively impact welfare.

Dissolved oxygen is a parameter influencing welfare, metabolism, robustness and fish performance. While both study species are considered tolerant of a wide range of dissolved oxygen, the report by the European Food Safety Authority (EFSA, 2008) set an oxygen saturation level of no less than 40% as a guideline.

As for salinity, both European sea bass and gilthead sea bream are regarded as euryhaline species, so gradual changes do not negatively impact their bodily welfare, whereas sudden ones may have a negative effect.

Especially when rearing in sea cages, water pH only shows minimal fluctuations that do not negatively impact the welfare of farmed fish. With particular reference to the two species in this study, guidelines report that pH values below 6.5 and above 8.5 are poor welfare conditions (EFSA, 2008). In normal rearing conditions in the sea, water pH range varies at around 8, while fluctuations are limited and do not have negative consequences for fish welfare.

Water turbidity refers to transparency, which is affected both by the presence of solid suspended particles and dissolved substances. It is not usually a problem in sea rearing conditions; the presence of turbidity in tank water may negatively impact fish wellbeing both directly and indirectly (e.g. in relation to increased bacterial density), and should be further investigated to establish its causes.

2.2 | Nutrition

Proper feeding is of primary importance to fish welfare. In intensive farming conditions the amount of feed is not usually an impediment to animal wellbeing. What may have a negative impact on animals, however, is feed quality and feed delivery management.

In the larval stage, following consumption of the yolk sac reserves, both European sea bass and gilthead sea bream are fed with live feed. For both species this contains enriched nauplii of *Artemia sp*. crustaceans and *Brachionus sp*. rotifers. There are also larval rearing protocols that include the additional provision of *Chlorella sp*. phytoplankton [the pseudo-green water method] (Papandroulakis *et al*., 2001). To improve larval survival and growth, these feeds are enriched with polyunsaturated fatty acids, phospholipids and amino acids. A gradual transition is then made from providing live feed to dry commercial feed, which is fully completed around 40-50 days after hatching, depending on the rearing protocol used.

The composition and raw materials in individual feed ingredients are of major importance in catering for fishes' biological needs. For instance, it has emerged that the balance between the quantity of protein and digestible energy in food is an important factor in growth and effective nutrient utilisation (Kousoulaki *et al.*, 2015). Beyond that, nutrition is the only source of specific nutrients such as certain amino acids, vitamins and minerals that fish cannot synthesize. Changes in the quantities of provided ingredients may lead to significant changes in fish physiology and immune condition (Sitjà-Bobadilla and Pérez-Sánchez, 1999; Azeredo *et al.*, 2017).

Apart from composition and quality, another significant factor is feed delivery management in relation to quantity and feeding frequency. Especially at feeding time, aggression in European sea bass and gilthead sea bream has been seen to increase when fish are given less than the recommended amount of feed (Andrew *et al.*, 2004; Oikonomidou *et al.*, 2019) and when it is delivered exclusively to the same area of the tank. Aggression is more intense in European sea bass than in gilthead sea bream (Oikonomidou *et al.*, 2019).

Regarding feeding frequency, two main husbandry factors can impact fish welfare: the temporal distribution of feed delivery over the day and scheduled or random feed delivery. Depending on developmental stage, physiological condition and environmental conditions, the digestion rate and feeding behaviour of each species should thus be borne in mind when estimating optimal feeding frequency – e.g. alterations in frequency and quantity in summer compared to winter. Lastly, it is important for feeding to take place according to a fixed timetable, as the fish will then know what time feed is delivered by following the innate biological rhythms in their physiology and behaviour that favour optimal feed utilisation (Sarà *et al.*, 2010; López-Olmeda *et al.*, 2012). Delivering food in a random fashion during the day may lead to an increase in blood cortisol concentrations and intense activity throughout the day when compared to fish fed at specific times (Sánchez *et al.*, 2009).

As far as nutrition is concerned, one important factor potentially impacting fish welfare is the fasting period animals may be subjected to prior to operations such as transport and harvesting. The consequences such intervals without feed may have depend on the duration of fasting, the physiological condition of fish and environmental parameters such as temperature. The European sea bass is a species known for its tolerance to short-term fasting, which can also rapidly return to normal weight levels after long-term fasting once feed delivery recommences (Rubio *et al.*, 2010; Chatzifotis *et al.*, 2011). Thus, short-term fasting does not appear to have negative consequences for fish survival, weight or physiological state (Rubio *et al.*, 2010; Caruso *et al.*, 2011). In instances of long-term fasting, however, the fish do lose weight (Caruso *et al.*, 2011; Chatzifotis *et al.*, 2011), while an increase in the concentration of lysozyme, a non-specific immune system parameter, has been observed in skin mucus (Caruso *et al.*, 2011). Recommencing feed delivery initially leads to increased consumption (Aranda *et al.*, 2001; Rubio *et al.*, 2010), ultimately resulting in weight recovery (Chatzifotis *et al.*, 2011).

In gilthead sea bream, even short-term (7-day) fasting may lead to weight loss, which is not observed in European sea bass (Ferreira Pinto *et al.*, 2007; Peres *et al.*, 2011). Furthermore, it is a species that does not show rapid weight recovery to the levels found in fish that have not undergone fasting (Eroldoğan *et al.*, 2006; Peres *et al.*, 2011; Skrzynska *et al.*, 2017), though a faster growth rate has been seen upon recommencement of feeding (Bavčević *et al.*, 2010). This fact highlights the special attention that should be paid during gilthead sea bream fasting periods, to avoid subjecting them to reduced states of welfare.

2.3 | Stocking density

Stocking density is a parameter potentially influencing fish welfare mainly in an indirect way, as it can lead to a significant deterioration in water quality and/or inhibit the expression of social relations and behaviours required for animal wellbeing. Density is usually expressed in terms of individuals per litre during larval rearing, and biomass per unit of volume (kg m^{-3}) in subsequent rearing phases. Knowledge surrounding optimal stocking density is incomplete, and stocking densities in fish cages are often selected in part empirically, varying from 10 to 20 kg m^{-3} or more (EFSA, 2008).

Rearing density is nevertheless a significant factor impacting welfare. From as early as larval rearing, its effects on fish performance have been observed, though not as much in the early larval stages as in subsequent phases of larviculture. Thus, on comparing densities of 20 and 200 individuals per litre at 14 days post fertilization (dpf) (Saillant *et al.*, 2003) and 50, 100, 150 and 200 individuals per litre up to 30 days post hatching (dph) (Hatziathanasiou *et al.*, 2002), no differences in fish performance were observed. At 49 dpf, however, fish at a stocking density of 20 individuals per litre were of a greater length than those at 200 individuals per litre (Saillant *et al.*, 2003). Furthermore, rearing at densities of 5, 10, 15 and 20 individuals per litre over the interval from 35 to 57 dph had a significant impact on the survival and performance of fry (Hatziathanasiou *et al.*, 2002). In particular, survival was greater at lower stocking density, while body weight and growth were greater at low and high density (Hatziathanasiou *et al.*, 2002). Similarly for European sea bream, a lower percentage of skeletal anomalies has been observed in rearing tanks with lower socking densities (16 vs. 100 individuals per litre) (Prestinicola *et al.*, 2013).

Most studies examining the effects of increased stocking density on fish performance and physiological condition after larval rearing concern land-based facilities and closed or open water circulation systems. In such cases, particularly regarding closed systems, it is difficult to distinguish the effect of stocking density from that of changes in water quality, and few studies have succeeded in separating the two.

Thus when the effects of increased stocking density (15, 30 and 45 kg m^{-3}) were studied in European sea bass over the course of 6 weeks, no differences in body weight or physiological parameters such as cortisol and glucose were observed, with the exception of elevated concentration of fatty acids when compared to the lowest rearing density. However, when the fish were exposed to an additional acute stress event, high density groups showed greater sensitivity, as reflected in elevated blood cortisol and fatty acid concentrations (Di Marco *et al.*, 2008).

In gilthead sea bream, different rearing densities (5, 10 and 20 kg m^{-3}) showed a negative correlation between specific growth rate (SGR%) and density after 30 or 62 test days, both in juvenile fish at a mean weight of ~10g (Sánchez-Muros *et al.*, 2017) and in individuals at a mean weight of ~270g after 63 days (Araújo-Luna *et al.*, 2018). The same result was also observed between stocking densities of 15 and 30 kg m^{-3} with gilthead sea bream at a mean weight of ~320g (Carbonara *et al.*, 2019a). In these studies, no significant differences in blood parameters (haematocrit, haemoglobin) or physiological parameters (cortisol, glucose) were observed between fish at the different rearing densities.

In studies where a deterioration in rearing water quality due to increased stocking density has been observed in parallel with greater density, the effects are even more intense. In such experimental designs, it has emerged that body growth and feed intake are lower at higher rearing densities of European sea bass (mean weight 72-180g), though no differences are apparent in physiological indices such as cortisol, glucose and haematocrit (Person-Le Ruyet and Le Bayon, 2009; Sammouth *et al.*, 2009; Santos *et al.*, 2010). On the other hand, in gilthead sea bream, increased stocking density accompanied by deterioration in rearing water quality acts on fish physiology and welfare, as manifested in elevated concentrations of cortisol, glucose, haematocrit and haemoglobin (Montero *et al.*, 1999; Sangiao-Alvarellos *et al.*, 2005; Mancera *et al.*, 2008; Arechavala-Lopez *et al.*, 2019) in fish at a mean weight of 7 to 400g. There are of course studies mentioning that these differences are only observed shortly after the trial commences, and that they then return to normal over a period of 2 to 7 days (Tort *et al.*, 1996; Rotllant *et al.*, 2000a; Barton *et al.*, 2005) after exposure to high stocking densities.

2.4 | Rearing system dimensions

The medium where fish are reared is a three-dimensional space, the parameters of which may influence living conditions. Knowledge about the effect of rearing system dimensions on fish welfare is very limited. For Mediterranean farmed species there are a few references concerning tank size during larval rearing, and one on European sea bass regarding the size of cages in sea farming. Thus, rearing larvae in tanks of differing volumes (2000, 500 and 40 litres) at the same density led to differences in larval growth and quality. Rearing in larger volumes resulted in the production of larval with a significantly higher growth rate, and a lower percentage of skeletal malformations and swim bladder problems (Lika *et al.*, 2015). Similarly, gilthead sea bream raised in larger tanks resulted in a lower percentage of skeletal malformations, leading the authors to recommend the use of tanks measuring at least 40 m^3 to reduce the emergence of defects (Prestinicola *et al.*, 2013).

As regards the size of cages during sea farming, rearing European sea bass (mean weight 130g) in cages of different volumes (1, 4, 45 and 252 m^3) at the same stocking densities resulted in significant differences in performance and welfare as regards physiological parameters between different experimental groups (Samaras *et al.*, 2017). In particular, growth rate was higher in the larger cages, while food conversion ratio (FCR) and cortisol concentration were lower, thus reflecting the fact that fish welfare is not only affected by stocking density, but – mainly – by available space, i.e. the usable volume in the rearing unit.

Lastly, one significant parameter regarding cage dimensions is depth. Fish are distributed at different depths in the cage depending on environmental rearing conditions (temperature, oxygen), husbandry practices (feed delivery, stress), physiological condition (ovulation period) and rearing at the larval stage (semi-intensive vs. intensive rearing) (Papandroulakis *et al.*, 2014). It is thus necessary to give cages sufficient depth to allow fish to express their behavioural needs.

2.5 | Hygiene and disease prevention

Maintaining hygienic conditions both in rearing medium and during handling procedures is important in preventing the emergence of infections and diseases borne by pathogens (parasites, bacteria, viruses and others) that may cause illnesses in fish. One further factor during larval rearing is the living feed delivered in the early developmental stages, via which larvae may become infected.

In farming conditions at sea, the fact that fish are exposed to pathogens means that higher rearing densities may favour the spread of disease. A series of preventive measures are implemented to prevent such phenomena, such as vaccination against specific pathogens, sampling to evaluate population health and maintaining hygienic conditions during operations. When fish are reared in tanks, e.g. broodfish, it is extremely important to maintain water quality at optimal levels and to observe strict hygiene rules so as to prevent diseases from emerging.

2.6 | Behaviour and social interactions

One of the basic concepts of welfare lies in the freedom of animals to express their behaviours. These include swimming, social interactions, exploration, periods of reduced activity and even reproductive behaviour in the case of mature breeding animals.

Although publications on available space and fish welfare are scanty, at least for European sea bass it has been observed that at the same stocking densities, physiological welfare indicators are better when the rearing unit volume is greater (Samaras *et al.*, 2017), thus permitting the freer expression of behaviours such as schooling and swimming.

With regard to social interactions, the two main behaviours that may cause or result from reduced welfare are aggression and competition. It has been reported that both European sea bass (Benhaïm *et al.*, 2011; Carbonara *et al.*, 2019a) and gilthead sea bream (Goldan *et al.*, 2003; Montero *et al.*, 2009; Castanheira *et al.*, 2013; Papadakis *et al.*, 2016) show aggressive behaviour, even forming hierarchical relations in groups of a few individuals under small-scale experimental rearing conditions. On the other hand, in floating units where thousands of fish are reared together, hierarchy relations are rarely established, and fish interactions are more opportunistic. Of course, in such cases food competition cannot be avoided; this may be indirect due to some fish reacting faster and more intensely to feed delivery (scramble competition), or directly expressed via aggression (EFSA, 2008; Attia *et al.*, 2012). Experiments with small populations in tanks have shown that aggressive behaviour may manifest itself during feeding, particularly in periods when feed intake is limited (Goldan *et al.*, 2003; Andrew *et al.*, 2004; Papadakis *et al.*, 2016; Oikonomidou *et al.*, 2019). Furthermore, there are indications that wider dispersed food delivery can reduce the frequency of aggressive behaviour in European sea bass, but not among gilthead sea bream, where it is comparatively lower overall (Oikonomidou *et al.*, 2019).

One further parameter of importance for welfare is respect for periods of reduced animal activity, which does of course presuppose knowledge of activity and rest periods in the animals under study. In general, fish appear to regulate their activity depending on feeding time (Velázquez *et al.*, 2004; Montoya *et al.*, 2010). Nevertheless, in cases studying autonomous feed provision via self-feeders, it has been observed that the species in question may be active both in the daytime and at night (Paspatis *et al.*, 2000; Rubio *et al.*, 2004; Velázquez *et al.*, 2004; Millot and Bégout, 2009). Furthermore, activity is heavily influenced by environmental conditions (especially temperature and photoperiod), with an increase in nocturnal activity during the coldest months (Rubio *et al.*, 2004; Velázquez *et al.*, 2004). Experimental data on the effects of disturbing rest periods in Mediterranean fish species are extremely sparse. Gilthead bream individuals manifesting daytime activity have been observed to show higher cortisol response to stress when provoked in the dark phase than in the light phase (Vera *et al.*, 2014). Similar results have been reported for other fishes, such as the zebrafish (Manuel *et al.*, 2014).

The normal commercial size for European sea bass and gilthead sea bream remains at 300-500g. However, changes in consumer habits (unwillingness to buy whole fish), the need for product diversification (e.g. fillets) and the higher sale price of larger-sized fish have led to the farming of European sea bass and gilthead sea bream at sizes in the order of 800g and above. In such instances both species may reach sexual maturity, with sex inversion from male to female occurring in gilthead sea bream in particular. Fish of this size thus manifest reproductive behaviour and spawn in their cages (Somarakis *et al.*, 2013). Regarding European sea bass, it has emerged that fish tend to gather more at the surface of cages at that time, with alterations also seen in their locomotive behaviour (Papandroulakis *et al.*, 2014). Consequently, it is important for fish wellbeing to take into account periods when reproductive behaviour is manifested and avoid operations or other stressful practices at that time.

Lastly, one further parameter potentially impacting fish behaviour in cages is the presence of predators – mainly marine animals such as dolphins, seals, birds and predatory fish. Although the primary consequence of predator attacks is fish death, there are others that may impact fish welfare. First comes wounding, and the potential consequent emergence of infections (Nash *et al*., 2000; Cooke, 2016). In addition, it has been observed that the presence of predators may provoke stress and changes in fish behaviour, particularly as regards swimming and nutrition (Nash *et al*., 2000; Güçlüsoy and Savas, 2003). The latter in particular may lead to reduced feeding and hence to suppressed body growth rate.

2.7 | Summary

- Good living conditions necessitate satisfying the biological needs of the species under study, including environmental and husbandry parameters.

- Environmental parameters such as temperature, oxygen, salinity, pH and turbidity mainly relate to water quality.

- Husbandry parameters include nutrition, both stocking density and rearing dimensions, hygiene and disease prevention.

- Lastly, good living conditions are typified by the free expression of individual and social behaviours in fish, and the prevention of displays of aggression between individuals.

3 | Indirect (environmental) Welfare Indicators

3.1 | Temperature

As ectotherms, fish are greatly affected by water temperature. Each species has a temperature range it can survive in, though that does not mean there are no negative impacts on animal welfare at the limits of that range. For European sea bass the range varies between 2-35°C (EFSA, 2008; Dülger *et al*., 2012), and for gilthead sea bream between 5-34°C (EFSA, 2008). That being said, there are also optimal temperature ranges for each species depending on developmental stage, which in the case of European sea bass vary between 10-20°C for eggs and larvae, and 8-28°C for larger individuals, and in that of gilthead seam bream 12-22°C and 8-30°C, respectively (EFSA, 2008).

Apart from absolute temperature level, the speed at which it changes also has an important role to play (Bertotto *et al*., 2011). Particularly during early developmental stages, when rearing is carried out in controlled land-based facilities, avoiding temperature changes is important. It is well known that rearing temperature in early developmental stages influences both performance and sex ratio in European sea bass, whereby a higher ratio of females emerges following larval rearing at low temperatures (13°C and 15°C versus 20°C) (Pavlidis *et al*., 2000; Piferrer *et al*., 2005). What is more, even 34 days post hatching, a temperature change from 15°C to 20°C during larval rearing can lead to a significant drop in the percentage of female individuals in the population (Koumoundouros, 2002). It has also been observed that sudden changes in the order of 0.5-1°C per hour cause mortality in larvae of both species (Madeira *et al*., 2016; Moyano *et al*., 2017) and should thus be avoided. Yet even among larger fish, marked temperature changes should be avoided (EFSA, 2008) both during transport (Samaras *et al*., unpublished data) and during raising in floating pens (Samaras *et al*., 2018a).

Measurement method

Temperature is one of the easily measurable indicators, and one very often incorporated into parameter monitoring routines at farms. It does not call for specialist equipment or trained staff. Recent years have seen the development of automatic recording systems at both floating and land-based installations, though purchase cost is high. In correctly assessing temperature it is vital to take measurements at different depths within cages.

Advantages of indicator

Temperature is easy and inexpensive to measure, while also being of vital importance to fish welfare and good performance. In addition, it also influences other indices such as oxygen saturation.

Disadvantages of indicator

As temperature is determined by the environment, there is no way of controlling and altering it during fish fattening in pens.

3.2 | Salinity

Both European sea bass and gilthead sea bream are euryhaline species, meaning that gradual changes in salinity are not anticipated to negatively impact their welfare. What may lead to reduced welfare or even mortality are sudden, extreme changes (Cataudella *et al*., 1991; Marino *et al*., 1994; Mabrouk and Nour, 2011). It is important to note that in European sea bass, tolerance to low salinities appears to be an individual characteristic (Thibaut *et al*., 2019) relating to population origin (Allegrucci *et al*., 1997).

Optimal performance in terms of growth and feed consumption has been recorded at salinity 30[1] for European sea bass and 18-28 for gilthead sea bream (Conides and Glamuzina, 2001). There are some reports of better growth in juvenile European sea bass at salinity 10 and 20 versus 30 and 40 (Eroldoğan and Kumlu, 2002), but the majority of studies indicate the opposite. Thus, low salinity at fresh water levels has been seen to negatively impact growth in European sea bass (Eroldtğan *et al*., 2004; Yilmaz *et al*., 2020); and when performances between low salinities (10 and 15) are compared, body growth is better at higher salinity (Goda *et al*., 2019). Fish at low salinities expend energy to maintain their osmotic balance (Masroor *et al*., 2018), particularly when fasting (Sinha *et al*., 2015), resulting in a negative impact on their welfare

Similarly, rearing gilthead sea bream at salinity 6 led to lower growth as compared with salinities 12 and 38, as well as lower osmotic concentration in the blood (Laiz-Carrión *et al*., 2005). On the other hand, studies carried out on larvae at 32 (Tandler *et al*., 1995) or 35 dph (Mohammed-Geba *et al*., 2016) showed better survival, growth and a higher percentage of individuals with a functional swim bladder at salinity 20 versus salinities 25 to 38. Lastly, negative consequences of salinity have also been observed in immune system indicators, both at low (12) and high (55) salinity compared to a control group (38) (Cuesta *et al*., 2005).

Measurement method

Salinity is yet another easily measurable indicator incorporated in parameter monitoring routines at fish farms. It does not require specialist equipment or trained staff. Correctly assessing it involves taking measurements from different cage depths.

Advantages of indicator

Easy and relatively inexpensive to measure.

Disadvantages of indicator

Significant and sudden fluctuations are not anticipated, meaning the indicator is rarely of use.

[1] Salinity expresses the amount of dissolved salts (in grammes) per kilo of seawater. The usual way of measuring it estimates "Practical Salinity", which depends on temperature and liquid pressure, and by definition does not have units of measurement. That is the reference method used here. For further information see https://www.nature.com/scitable/knowledge/library/key-physical-variables-in-the-ocean-temperature-102805293/

3.3 | Oxygen

The concentration of dissolved oxygen in water is a crucial parameter for fish welfare, as it defines the available oxygen for respiration. The effect of oxygen should of course always be monitored in relation to other environmental parameters, such as temperature and salinity (Claireaux and Lagardère, 1999), as well as the animals' energy demands (Claireaux and Lagardère, 1999; Pichavant *et al*., 2001).

Exposure to hypoxia conditions (40% saturation) during larval rearing negatively impacts welfare, as reflected in reduced growth rate (Vanderplancke *et al*., 2015; Cadiz *et al*., 2018a), lower toleration to new low oxygen events (Cadiz *et al*., 2018a), as well as lower aerobic activity capacity in later life stages as juvenile and adult individuals (Zambonino-Infante *et al*., 2017).

In subsequent developmental stages, both European sea bass and gilthead sea bream are regarded as species with high hypoxia tolerance, and welfare problems are only observed at low oxygen saturation levels (Thetmeyer *et al*., 1999; Pichavant *et al*., 2001; Araújo-Luna *et al*., 2018). In particular, a small drop in oxygen does not appear to have any negative effects on European sea bass as regards either oxygen consumption capacity (Claireaux and Lagardère, 1999) or physiological welfare indicators (Pichavant *et al*., 2001). Nevertheless, exposure to mild hypoxia (~60%) does appear to negatively affect the immune system of fishes (Cecchini and Saroglia, 2002). Lastly, in cases where dissolved oxygen is reduced to values approaching 40%, negative effects are seen both in aerobic activity capacity (Claireaux and Lagardère, 1999) and feed intake and growth (Thetmeyer *et al*., 1999; Pichavant *et al*., 2001; Cadiz *et al*., 2018b).

In gilthead sea bream, exposure to oxygen saturation of 40-60% did not lead to differences in growth when compared to a control group (80-100%), but to an elevated percentage of gill deterioration, thus signalling reduced welfare, which was not observed at saturations of 60-80% (Araújo-Luna *et al*., 2018).

Recent research in Greece on oxygen concentrations in European sea bass farming cages showed that in the summer months, oxygen saturation may approach the low levels proposed by the European Food Safety Authority (EFSA, 2008) as a guideline for the emergence of welfare problems (Makridis *et al*., 2018). It is thus extremely important for preventive measures such as maintaining net cleanliness to be taken, so as to avoid such phenomena.

Despite the resilience of both species to low oxygen concentrations, negative effects are observed when changes in saturation are acute and marked. In particular, a sudden drop in oxygen levels by around 80% led to loss of consciousness and death in both species (Claireaux and Chabot, 2016; Magnoni *et al*., 2017). Changes of this type have also been seen to affect physiological welfare indicators such as cortisol, glucose and lactate (Magnoni *et al*., 2017; Martos-Sitcha *et al*., 2019).

Apart from decreases in dissolved oxygen, welfare problems may also be caused by increases (hyperoxia), particularly in land-based installations with an oxygen supply. Moderate hyperoxia in the order of 150% has been observed not to cause mortalities or significant changes in growth and the immune system of European sea bass (Cecchini and Caputo, 2003; Lemarié *et al*., 2011). Nevertheless, marked hypoxia (over 200% saturation) negatively affects survival, growth and ionic balance in the blood (Lemarié *et al*., 2011), particularly when accompanied by hypercapnia (Petochi *et al*., 2011). Long-term exposure to oxygen-supersaturated rearing water has also been reported as causing gas bubble disease and observed mortalities.

Measurement method

Oxygen concentration and saturation are measured using oxygen meters. As the levels may vary at different points in fish cages or tanks, it is important to take measurement at several points, placing emphasis on areas where conditions are anticipated to be worst (e.g. at the water outflow in an open circuit tank). Lastly, automated continuous recording systems have been developed for use on floating and land-based installations, though this adds significantly to the cost of monitoring the indicator.

Advantages of indicator

This is one of the most important parameters in fish welfare. It is easy to measure when the appropriate instruments are available.

Disadvantage of indicator

The fact that differences that may exist in a single rearing unit necessitates measuring several points, with the added risk that points low in saturation may not be assessed.

3.4 | pH

pH is a qualitative characteristic of water that varies little in open sea rearing conditions. That being said, in cases where rearing occurs in land-based tanks, pH has to be monitored systematically as it may be lower than in the sea and change relatively more markedly.

In the few studies examining the effects of low pH (in the order of 7.6-7.8) during larval rearing, conflicting results have been obtained. In particular, some studies show no observable effect of low pH on growth, development or larval swimming behaviour (Duteil *et al.*, 2016; Cominassi *et al.*, 2019), while rearing at pH 7.7 versus 7.9 or 7.5 showed higher survival, but lower body growth at 35 dph (Crespel *et al.*, 2017). Lastly, in another study, rearing at pH 7.8 promoted faster growth at 19°C but not at 17°C (Pope *et al.*, 2014). In gilthead sea bream, on the other hand, studies agree that lower pH (between 6.0 and 7.5 compared to 8.0) negatively impact hatching, survival, growth and skeletal malformations (Basallote *et al.*, 2012; Pimentel *et al.*, 2016).

In larger individuals of European sea bass, increase in blood biochemical stress indicators (glucose and lactose) was observed on exposure to pH 7.7 versus 8.2 (Shrivastava *et al.*, 2019). No differences in growth were noted in gilthead sea bream over a pH range from 8.1 to 7.5 (Réveillac *et al.*, 2015), although in another study metabolic changes (metabolism of glycogen and fats, glycolysis) were reported in fish exposed to pH 7.5 versus 8.0 (Araújo *et al.*, 2018).

It is worth noting that the majority of the above experimental studies use carbon dioxide as a means of reducing pH, so care is required in evaluating the results as regards identifying the cause of observed changes. For that reason, the present guide follows the guidelines in the EFSA report (2008), which mention that for both species, pH values of below 6.5 or above 8.5 are poor living conditions, whereas gradual changes are not anticipated to negatively affect welfare.

Measurement method

pH is an easily measurable indicator when appropriate portable meters are used. As with the remaining environmental parameters, it is important to take measurements from different areas in the rearing installation.

Advantages of indicator

Easy to measure.

Disadvantages of indicator

Often shows very slight changes, thus calling for use in combination with other welfare indicators.

3.5 | Turbidity

Water turbidity is defined as "the disturbance or reduction in light transmittance in water resulting from suspended, colloidal or dissolved matter or the presence of planktonic organisms" (Eleftheriou, 1998). It is a parameter that does not usually concern floating units in the sea, though it may influence fish living conditions mainly in combination with other parameters (e.g. reduced oxygen, increased bacterial density).

Measurement method

Turbidity can be directly estimated by sight. For greater accuracy it can be quantified using a secchi disc or digital meters.

Advantages of indicator

May be an early indication of deterioration in rearing water quality, as well as of changes in other parameters such as dissolved oxygen and pathogen growth.

Disadvantages of indicator

As a general measurement it does not pinpoint the nature of the problem, impeding the adoption of specific treatment measures.

3.6 | Stocking density

Stocking density (rearing density) can affect fish living conditions both directly (mainly in terms of the manifestation of natural behaviours) and indirectly (deterioration in water quality, hygiene, stress, health). Many of the negative effects of increased rearing density on welfare may thus not originate in stocking density per se, but rather in the changes it brings about. As the interaction of these factors in combination with rearing technology makes it difficult to establish optimal stocking densities, values of between 10-20 kg m^{-3} are generally used on the basis of empirical data supported by scientific literature.

During the larval rearing stage of both species, it has been observed that both lower rearing densities (Hatziathanasiou *et al*., 2002; Saillant *et al*., 2003; Prestinicola *et al*., 2013) and larger rearing volumes (Prestinicola *et al*., 2013; Lika *et al*., 2015) favour survival, body growth and performance, while reducing the incidence of skeletal malformations.

Furthermore, for European sea bass (Person-Le Ruyet and Le Bayon, 2009; Sammouth *et al*., 2009; Santos *et al*., 2010) and particularly for gilthead sea bream, it has been observed that high stocking densities during pre- and on- growing lead to a reduction in body growth rate, mainly when paralleled by water quality deterioration. More marked fin damage is also reported for European sea bass when reared at high densities (Person-Le Ruyet and Le Bayon, 2009).

As far as welfare indicators regarding physiology (e.g. cortisol and glucose) are concerned, it has been observed that European sea bass raised at high stocking densities (36 to 75 kg m^{-3}) show difficulty in coping with an additional stressful operation, as reflected in elevated cortisol levels (Di Marco *et al*., 2008; Lupatsch *et al*., 2010; Santos *et al*., 2010). On the other hand, negative effects on welfare indicators such as higher cortisol, glucose, haematocrit and haemoglobin have been observed in gilthead sea bream at high stocking densities (20 to 70 kgm^{-3}), both in the long term (Montero *et al*., 1999; Sangiao-Alvarellos *et al*., 2005; Mancera *et al*., 2008; Arechavala-Lopez *et al*., 2019) and in the short term, with recovery to earlier levels after 2-7 days (Tort *et al*., 1996; Rotllant *et al*., 2000a; Barton *et al*., 2005). It is of course important to mention that the majority of the above studies on both species were carried out in open or closed-circuit tanks, and not in floating marine installations.

Research into European sea bass (Samaras *et al*., 2017) and other species such as marbled spinefoot (*Siganus rivulatus*) (Bukhari, 2005) have shown that in addition to density, a significant role in fish welfare is played by "available volume", being the volume free for fish to manifest natural behaviours. Thus, expressing volume as kg m^{-3} is not the only factor affecting the living conditions of fish, as the volume of water available for them to express their behavioural and social needs should also be taken into consideration (Samaras *et al*., 2017). At present there is no evaluated indicator for assessing this characteristic.

Measurement method

During larval rearing, density is defined as the number of individuals per litre and is determined by egg stocking processes. As fish are especially sensitive in these stages, operations to change densities are not undertaken. When rearing larger individuals, density is defined as biomass per unit of volume (usually in kg m^{-3}). Correctly estimating stocking densities in sea cages involves keeping records of the number of individuals, taking into account mortalities and regular weight measuring samples to adjust biomass. New technologies such as submersible camera drones, automated software and hydroacoustic systems may assist in the more accurate, less invasive and more automated estimation of stocking densities.

Advantages of indicator

Stocking density is an important parameter describing living conditions, which is easily calculable when accurate data is available on the number of fish, their mean weight and rearing volume.

Disadvantages of indicator

May influence welfare indirectly and must therefore be evaluated in parallel with other indicators such as water quality. As the absolute value of this indicator is not a reliable parameter, it should be co-evaluated with the fishes' way of life, developmental stage, and behaviour, as well as rearing volume and technology. In some cases, it may change markedly and instantly, e.g. during feeding, when density increases dramatically in the area where feed is administered. Lastly, where no accurate data is available to calculate rearing density, the assessment may be erroneous, differing considerably from the actual level.

3.7 | Lighting

Lighting at fish cages in the sea is natural, and thus not anticipated to impact welfare. Nevertheless, there are periods in fish's lives when they are exposed to artificial lighting, e.g. larval rearing, pre-growing and brood stock. In these cases, lighting is a complex factor described in terms of its intensity and quality (spectrum), as well as its periodicity (photoperiod). It has emerged that all three parameters may impact fish wellbeing (EFSA, 2008).

With regard to periodicity, lighting is avoided during the yolk-sac larval stages of both European sea bass and gilthead sea bream (EFSA, 2008), because although growth is promoted in continuous lighting conditions, unsatisfactory yolk sac consumption, jaw deformities and swim bladder problems are observed at the same time (Villamizar *et al*., 2011). In subsequent developmental stages light is an important factor in initiating predatory activity, while photoperiods of long duration appear to favour growth (EFSA, 2008).

As far as light intensity is concerned, according to the EFSA report (2008), intensity should be low (100-500 lux) during the larval rearing of European sea bass, whereas gilthead sea bream require stronger light (1.000-3.000 lux). Earlier studies on European sea bass give similar results (Barahona-Fernandes, 1979; Johnson and Katavic, 1984), though later ones do not appear to confirm this (Cuvier-Péres *et al*., 2001), even showing better growth though at increased mortality under high intensity lightings (Yan *et al*., 2019). Differences in larval performance also arise in rearing under a different light spectrum, showing better growth and survival in blue or white lighting conditions (Villamizar *et al*., 2011).

In subsequent developmental stages lighting may have an important role to play in performance, physiology and welfare (Ginés *et al*., 2004; Karakatsouli *et al*., 2007; Vera *et al*., 2010; Vardar and Yildirim, 2012; Yildirim and Vardar, 2015), as well as in the unimpeded functioning of fishes' biological rhythms (Cerdá-Reverter *et al*., 1998; Sánchez-Vázquez *et al*., 1998; Gómez-Milán *et al*., 2011). That being said, under rearing conditions in floating sea pens, light is as a rule natural and this is not a cause for concern as regards fish welfare. The use of artificial lighting to provide constant light was investigated in the past, with a view to preventing the early sexual maturation of males in European sea bass (Rodríguez *et al*., 2005; Felip *et al*., 2008). The consequences this had for welfare were not studied thoroughly, and the practice tends not to be used any more. Changes in lighting (mainly photoperiod) is employed in broodfish rearing in land-based installations to provoke spawning (Zanuy *et al*., 1986; Carrillo *et al*., 1989). Lighting management is used in conjunction with temperature to achieve out-of-season spawning to produce fry all year round.

Measurement method

The application of this indicator is limited under rearing conditions in floating sea pens. It is particularly important during larval rearing and in spawning operations, where lighting is artificial. In these cases, the period, intensity and spectrum of lighting must be known and monitorable.

Advantages of indicator

Lighting is a significant parameter in larval rearing and spawning operations. In cases were artificial lighting is used, adjusting parameters to desired levels is not anticipated to be altered by anything other than technical problems.

Disadvantages of indicator

Lighting is not readily controllable when rearing in fish pens in the sea. In cases where artificial light is used, dispersal to the entire volume of tanks or between tanks may not be the same; detailed study and proper installations are required to avoid such instances.

3.8 | Summary

- Indirect (environmental) welfare indicators offer useful information on fish rearing conditions. Many of them (e.g. temperature, salinity and oxygen) are regularly monitored by fish farm staff, as they are crucially important to fish wellbeing.
- The various indicators differ from others both in terms of measurement and certain advantages and disadvantages. Furthermore, there are indicators which depend on each other, e.g. oxygen saturation is dependent on temperature. For that reason, concurrent assessment of more than one indicator is required to gain a fuller picture of welfare conditions.
- Several environmental indicators (e.g. temperature, salinity, pH, turbidity) are controllable under rearing conditions in land-based installations, but not in floating rearing pens.
- On the other hand, other indirect indicators are determined by rearing strategy, such as stocking density, the rearing medium volume and lighting.

4 | Biological Welfare Indicators

4.1 | Mortality

Mortality rate and extent is the most commonly used biological welfare indicator. It is a simply and immediately observable indicator, whereby increased rate indicates the existence of a serious problem in fish rearing conditions. In any case, it is important to establish anticipated mortality ceilings, so as to better assess whether and how observed mortality relates to reduced fish welfare.

To date there are no published natural mortality curves for European sea bass and gilthead sea bream, as there is e.g. for Atlantic salmon (Soares *et al.*, 2011), which could be used as a reference base for assessing observed mortality at a specific area, time and production phase. Such curves do of course suffer from the weakness of generalisation, as the areas where there are fish farms in Greece differ from each other geographically, oceanographically etc. Thus, the most advisable method is to have curves for each farm from mortality data in previous years, on the basis of which acceptable or unacceptable mortality rate ceilings can be established.

Both long- and short-term mortality can serve as welfare indicators. Sudden, short-term increase in mortality provides a snapshot of acute reduced welfare, the causes of which should be investigated. Thus, although recording a small number of dead individuals every day is no reason for concern, records of dozens of dead fish should serve as a sign to further investigate fish welfare conditions. The severity of long-term mortality is harder to assess, as there are no mortality curves for Mediterranean species setting limits on cumulative mortality not reflective of reduced welfare conditions. Records of cumulative mortality in the order of 35% in one cage could be regarded as a practical limit that ought to serve as a warning signal (personal communication with producers).

Measurement method

Evaluating mortality involves collecting and counting dead animals on a daily basis. In periods of high mortality, the cause of death should be determined (e.g. disease, environmental parameters) so as to evaluate the impact on welfare, and above all so that steps can be taken to mitigate the phenomenon and avert it.

Advantages of indicator

Assessing the indicator is simple, and already part of the routine procedures on fish farms. Although mortality is usually the end result of reduced welfare, in cases such as disease onset it may be a signal that the problem should be diagnosed and dealt with.

Disadvantages of indicator

The main disadvantage of this indicator is that it usually represents the end result of unacceptable welfare conditions. Reversing the immediate consequences is thus difficult.

4.2 | Health

Health is one of the most important welfare indicators, as the presence of a disease is a sign of poor living conditions. This indicator includes numerous clinical circumstances, such as natural pathogenesis, communicable diseases and ectoparasites, and can also be evaluated on many levels, from macroscopic examination to specialist laboratory analysis. This complexity means that promptly establishing cause and effect relationships to tackle disease is often difficult.

According to the results of research into Mediterranean fish farming, plus specialist opinion in the field of fish pathology, the main diseases faced by sea-reared fish in Greece are viral encephalopathy and retinopathy/viral nervous necrosis (VER/VNN)], vibriosis, photobacteriosis, isopod infestation and flatworm infestation in the gills (Vendramin *et al.*, 2016). These diseases have different clinical characteristics, different prevention and treatment methods and of course differences in virulence and contagiousness. The emergence of disease is a natural process, as pathogens are encountered in the sea as a matter of course. There are some fish farming practices that may favour the emergence and above all the spread of diseases, such as high stocking density, poor water circulation through nets and crowding. Furthermore, practices potentially leading to fish injuries such as loss of scales may increase the likelihood of pathogens entering an organism.

Measurement method

Monitoring fish health is a routine procedure that can prevent mass disease outbreaks. This may include everything from a simple macroscopic examination to carrying out necropsies for laboratory evaluation of the situation. Reaching conclusions requires specialised, accredited staff.

Advantages of indicator

Particularly in high risk periods, regular checks may prevent the emergence of a disease if precautionary measures are taken.

Disadvantages of indicator

On the other hand, diseases are only recorded once they have emerged, even if this is before they reach levels that can threaten a population. Furthermore, the absence of disease is not by definition synonymous with good welfare conditions, rendering the additional use of other welfare indicators necessary.

4.3 | External appearance and injuries

The external appearance of the skin, fins and other structures such as the mouth and eyes are important welfare indicators, while also affecting consumers' idea of the end product where imperfections are noticeable. In addition to the skeletal defects covered in the following section, the causes of these imperfections vary from injuries (in the main) to diseases including parasitosis and poor nutritional state in fish (Noble *et al.*, 2012).

The most frequently observed skin problems involve loss of scales or skin parts, bleeding and ulcers. One major cause of these conditions are injuries, which may also be symptoms of diseases such as vibriosis. Injuries may be due to rearing structures including nets and tank walls, as well as operations such as netting, exposure to air, transport etc.

Injuries to fins occur for the reasons described above. In particular, two important factors affecting injuries of this type are high stocking density (Person-Le Ruyet and Le Bayon, 2009; Araújo-Luna *et al.*, 2018), and inadequate fish feeding, which may lead to heightened aggression (Goldan *et al.*, 2003; Andrew *et al.*, 2004; Papadakis *et al.*, 2016; Oikonomidou *et al.*, 2019).

Skull structures such as the mouth and eyes are also sites for monitoring the external appearance of fish (Noble *et al.*, 2012). Particularly in the case of eyes, the most frequently observed complication is exophthalmia, though this has various causes – apart from injury, it may be due to infectious diseases or the presence of gases in the blood (Noble *et al.*, 2012). Beyond exophthalmia, the eyes may exhibit bleeding or cataracts, the latter being more common in gilthead sea bream than in European sea bass (Bjerkås *et al.*, 2000). Mouth and eye problems are anticipated to negatively impact fish welfare, as they affect feeding capacity and the manifestation of natural behaviours, and may even cause death (Noble *et al.*, 2012).

Measurement method

Particularly as regards the skin, injuries can be recorded either at stock level, e.g. when scales or blood are seen in the water, or via the macroscopic examination of individual fish. Macroscopic, individual examination is required to monitor other cases. Injuries may be recorded as "present" or "non-present", with systems for evaluating injury severity such as the five-grade scale for European sea bass fins proposed by Ruyet and Bayon (2009) being used for more detailed recording. There is currently no published scale classifying all of the potential injuries mentioned above.

Advantages of indicator

An increase in injury incidence points to poor living conditions, and is an extremely useful indicator for evaluating practices where operations are carried out on animals, such as crowding, exposure to air, transport, preventive treatment etc.

Disadvantages of indicator

Identifying the cause of imperfect fish appearance such as exophthalmia is not always easy. When an increase in fish injury incidence is observed, it means that living conditions have already declined.

4.4 | Malformations[2]

Skeletal malformations are deformities of the skeleton such as the skull, including the jaws, the spine, the peripheral bones and the gill cover bones. High rates appear during larval rearing, and usually accompany individuals throughout their life. Deformities of this type are also encountered in wild individuals, though at lower rates than in farmed ones (Sola *et al.*, 1998; Sfakianakis *et al.*, 2013).

Fish with skeletal malformations are a problem for producers, as they are not selected by customers. Yet beyond this, malformations may also be problematic for fish welfare. In particular, although it is now known whether skeletal deformities cause fish pain (Branson and Turnbull, 2008), it goes without saying that fish with such malformations may show higher mortality (Koumoundouros, 2002) and experience difficulty feeding and breathing, especially in the case of skull deformities (Noble *et al.*, 2012), and lower swimming performance, particularly as regards spinal deformities (Basaran *et al.*, 2007).

Factors influencing the emergence of skeletal deformities are both environmental and genetic. Thus, it has been seen that larval rearing temperature can markedly affect the incidence of skull and spinal malformations in both species (Sfakianakis *et al.*, 2006; Georgakopoulou *et al.*, 2007, 2010). Current speed also impacts the emergence of spinal deformities, mainly in the form of lordosis (Chatain, 1994; Divanach *et al.*, 1997). In addition, both small available volumes in rearing units and high rearing densities appear to lead to an increase in the percentage of individuals with skeletal deformities (Can, 2013; Prestinicola *et al.*, 2013; Lika *et al.*, 2015). Lastly, poor nutritional condition in broodfish appears to lead to an increase in the percentage of deformed individuals among their offspring (Cerdá *et al.*, 1994), while larval nutrition affects the emergence of malformations, reducing their emergence when larvae have a diet rich in amino acids (Cahu *et al.*, 2003a) and phospholipids (Cahu *et al.*, 2003b).

[2] Issues concerning congenital anomalies and physical defects will be discussed in a relevant forthcoming publication.

As far as the influence of genetic factors is concerned, it has emerged that particularly as regards the spine, skeletal deformities show medium to high heredity in both European sea bass (Bardon *et al.*, 2009; Karahan *et al.*, 2013) and gilthead sea bream (García-Celdrán *et al.*, 2015; Lee-Montero *et al.*, 2015; Negrín-Báez *et al.*, 2015a). Genetic loci associated with the emergence of skeletal malformations have been identified in gilthead sea bream (Negrín-Báez *et al.*, 2015b).

Measurement method

Skeletal deformities are recorded either by visual observation (mainly in large individuals), photography following staining (mainly in larvae) and X-ray imaging. Skeletal malformations range very widely, and are thus usually classified into groups depending on their type and/or severity.

Advantages of indicator

Relatively easy to record, especially in large individuals.

Disadvantages of indicator

Particularly during visual observation, the issue of observer objectivity may intrude. More than one observer is thus required for reliable results. Additionally, due to the different causes potentially responsible for skeletal deformities, establishing cause-effect relationships is difficult.

4.5 | Nutrition and appetite

Nutrition and appetite in particular can serve as a welfare indicator, despite the fact that identifying the causes of anorexia is difficult. It goes without saying that stress and poor living conditions negatively impact fish nutrition and feeding (Pichavant *et al.*, 2001; Rubio *et al.*, 2010; Leal *et al.*, 2011). Even simple husbandry practices such as tank cleaning have been observed to cause abstinence from food or reduced appetite, particularly in European sea bass (Rubio *et al.*, 2010), while gilthead sea bream appear more resilient to disturbance (Samaras *et al.*, 2018b).

That being said, appetite is influenced by many other parameters, such as genetic predisposition, physiological condition of the body, stomach fullness at feeding time, feed composition, environmental conditions and also possible competition between individuals. Temperature appears to pay a leading role in appetite regulation, increasing food consumption when it rises (Russell *et al.*, 1996; Peres and Oliva-Teles, 1999a), before plateauing at high temperatures of 28-30°C (Person-LeRuyet *et al.*, 2004). Appetite may also be influenced by available oxygen concentration (Pichavant *et al.*, 2001) and photoperiod (Ginés *et al.*, 2004).

It is thus obvious that reduced feed consumption is not necessarily associated with poor living conditions. Likewise, it is important to stress that in their natural environment, fish may spend intervals without feeding. As a consequence, reduced food intake does not automatically mean that fish welfare is being affected (Caruso *et al.*, 2011; Chatzifotis *et al.*, 2011; Peres *et al.*, 2011; Skrzynska *et al.*, 2017). Providing less than the desired amount of feed should nonetheless be avoided in intensive rearing conditions, as it can lead to heightened competition between individuals (Goldan *et al.*, 2003; Andrew *et al.*, 2004; Papadakis *et al.*, 2016; Oikonomidou *et al.*, 2019) and indirectly to reduced welfare. On the other hand, providing more than the required quantity of feed leads to producers wasting resources. It is thus necessary to monitor feed delivery and adjust it according to fish demands. This is far from easy in practice and has been the object of intensive study, with considerable research activity aimed at utilising new technologies in fish farming, such as camera monitoring, implementing the internet of things and artificial intelligence.

Lastly, feed composition may cause fish welfare problems via the emergence of “nutritional diseases” such as lipidosis, mainly of the liver (Amar & Lavilla-Pitogo 2004; Caballero *et al.*, 2004), as well as deficiencies in vital fatty acids (Montero *et al.*, 2004; Skalli *et al.*, 2006), amino acids, vitamins and inorganic elements such as phosphorus (Kousoulaki *et al.*, 2015).

Measurement method

Farms quite often keep data on daily feed supply to fish cages, which can serve as an indication of appetite when expressed as a function of the biomass or number of individuals in the population. Particularly in instances of hand feeding, when staff provide feed to the point of satiation, these data are of especial value in comparing the appetite observed in a cage at a given moment in time. It is anticipated that new technologies such as underwater cameras and automatic analysis systems will greatly assist in this direction.

Advantages of indicator

Appetite assessment is an easily measurable and non-invasive indicator which does not require specialist staff. Furthermore, it can serve as a direct indication of reduced welfare, prompting more thorough inspection to pinpoint and treat the causes. Lastly, it can be utilised as a retrospective monitoring indicator for the effect of an operation, since after stress fish show zero or reduced appetite for food, the duration of which, in European sea bass, depends on the severity of the operation (Rubio *et al.*, 2010).

Disadvantages of indicator

The two basic weaknesses of this indicator are the fact that appetite may be influenced by factors other than reduced welfare, and the difficultly in precisely assessing and comparing it to “anticipated” or “normal levels”.

4.6 | Body Growth

Body growth is a commonly used indicator, as a healthy fish that feeds properly is expected to increase in size. As in the case of nutrition, body growth may be affected by several parameters other than poor welfare conditions; growth directly depends on the genetic origin of stock, developmental stage, temperature and the fish's physiological condition.

Nevertheless, factors capable of negatively impacting body growth do exist. Among these, water quality (Pichavant *et al*., 2001; Cadiz *et al*., 2018a; Yilmaz *et al*., 2020), feed delivery method and nutritional value (Montero *et al*., 2004; Skalli *et al*., 2006; Kousoulaki *et al*., 2015), stress (Rubio *et al*., 2010; Samaras *et al*., 2018b), rearing density (Sammouth *et al*., 2009; Santos *et al*., 2010; Sánchez-Muros *et al*., 2017; Araújo-Luna *et al*., 2018; Carbonara *et al*., 2019b) and health have a considerable effect on fish growth. Reduced body growth rate should serve as an indication to producers to thoroughly investigate, in combination with other welfare indicators, whether and to what extent fish are encountering poor living conditions.

Measurement method

In farmed fish, body growth usually refers to increases in body weight and, to a lesser degree, body length. There are several ways of expressing body growth, the most commonly employed one being "specific growth rate" (SGR%), which gives the percentage increase in weight per day over a fixed time interval. Reliable weight data are required at all events; obtaining them using normal sampling measurements is difficult in instances where thousands of fish are being raised in floating cages. New technologies to assess weight using non-invasive measurement methods such as submersible camera drones with appropriate software have been developed in recent years and are expected to be piloted.

Advantages of indicator

Growth is an indicator already being monitored at many fish farms. Reduction in growth rate may be associated with reduced welfare, thus serving as a direct warning sign.

Disadvantages of indicator

As mentioned, assessing this indicator reliably and in a manner truly representative of the entire population is difficult in rearing conditions with thousands of fish in floating cages. Furthermore, assessing whether and to what degree the observed fish growth rate is low requires data from previous years in every area for comparative monitoring purposes.

4.7 | Respiration rate

Oxygen demands increase when organisms are under stress. This may be reflected in a rapid increase in respiration rate. In particular, breathing rate is used as an easily observable stress indicator in fish (Poulton *et al*., 2017; Spiga *et al*., 2017). Of course, respiration rate does not only change under stress conditions; it may also reflect alterations in environmental conditions such as temperature, salinity and available oxygen (Dalla Via *et al*., 1998; Claireaux and Lagardère, 1999), as well as an animal's nutritional state and energy demands (Peixoto *et al*., 2016).

Measurement method

Assessing this indicator involves recording the number of respiratory movements of the opercular bone per unit of time. Accurately quantifying it is difficult in fish farming conditions; it is more easily approximated by farm staff as a comparative estimation of respiration rate during daily routine practices, with marked increases in it being used as an advance warning for more detailed monitoring of other finer indicators. More accurate assessment of the indicator can be carried out at laboratory level, via individual measurements. At all events, when measuring either accurately or relatively it is vital that the fish be in a calm state, showing little movement.

Advantages of indicator

Respiration rate is an important indicator in welfare assessment, which can be regularly monitored by farm staff during routine practices. It is cost free.

Disadvantages of indicator

Accurately recording is difficult in farm conditions with thousands of fish per cage. Doing so requires individually monitoring animals, though this practice is anticipated to cause stress and, by extension, an increase in respiration rate. It is also affected by several parameters such as natural, seasonal temperature increases, and does not always imply a deterioration in living conditions.

4.8 | Maturation and spawning

This indicator mainly concerns two types of rearing: brood stock and fish farmed for sale as high commercial weight class products.

Particularly in the case of brood stock management, a series of practices may negatively impact welfare. The main ones carried out in brood stock units are:

1. Individual fish tagging (especially in genetic selection programmes).
2. Exerting light pressure on the abdominals walls (stripping) and/or biopsies to determine sex/gonad maturation stage.
3. Alteration in environmental parameters, mainly photoperiod and/or temperature, for out-of-season egg production.
4. Hormone therapy to induce spawning.
5. Genetic selection.
6. In the case of gilthead sea bream, changes in social relations by adding younger males to stock.

Broodfish stocks are often marked with individual PIT (Passive Integrated Transponder) tags for individual identification purposes. This practice is particularly widespread in stocks used in genetic selection programmes, and where brood fish are selected on the basis of their genetic and phenotype characteristics. It should be carried out under anaesthesia.

Whenever necessary, sex ratio monitoring is carried out by checking the percentage of individuals that release milt during stripping. Gilthead sea bream show a peculiarity with regard to this characteristic, since as a protandrous species they initially mature as males, with sex inversion to female being observed in certain individuals from the second and, in the main, third year of life. The technique should be carried out under anaesthesia.

More detailed monitoring of reproductive maturation can additionally be carried out via hormone analysis (following blood sampling) as well as biopsies, mainly on female individuals, so as to accurately record gonad maturation stage. Recording this indicator is important because sexual maturation may trigger behavioural changes, particularly in spawning periods, during which husbandry practices should be changed accordingly so as to avoid unnecessary disturbances to fish.

Changes to photoperiod and/or temperature are commonly used to induce out-of-season egg laying (Carrillo *et al*., 1989; Mañanós *et al*., 1997; Kissil *et al*., 2001). Both the method and intensity of photoperiod changes and the quality of lighting used can also impact animal welfare, e.g. due to growth and feed consumption (Kissil *et al*., 2001; Ginés *et al*., 2003).

The use of hormone therapies to induce and synchronise maturation and spawning is likewise common (Zohar and Gordin, 1979; Mylonas *et al*., 2003). This involves operations such as catching, exposure to air and anaesthesia. It follows that supplementary monitoring of other welfare indicators as described above is also necessary. Lastly, once the procedure is completed, maturation stage and successful or unsuccessful spawing are monitored and recorded.

Genetic selection programmes aimed at improving growth rate and/or disease resistance may also present a challenge for animal welfare warranting further study (Hastein, 2004; Humane Society International, 2012). Furthermore, selection for desired characteristics may lead to co-selection of undesirable traits with an impact on the welfare of offspring.

Since gilthead bream is a hermaphrodite protandrous species, in which sex is also influenced by social parameters, the entry of young males into stock may alter social balances, leading to an increase in sex inversion in older male individuals (FAO, 2005). Furthermore, the existence of more females delays sex inversion in younger males.

Finally, it is worth noting that techniques that cause polyploidy or sterility are not employed in Greek aquaculture. These are aimed at improving growth rate but are also accompanied by challenges to animal welfare (Hastein, 2004).

Measuring method

The commonest way of monitoring sex in mature reproductive individuals is by recording the proportion of them that secrete milt following stripping. More detailed examination can be carried out by laboratory analyses such as biopsies or hormone tests. Furthermore, correctly assessing the practices implemented on brood fish involves parallel measurement of other indirect (e.g. environmental) and biological indicators (such as growth, behaviour, health), detailed description of which is provided in Chapter 6.

Advantages of indicator

Knowledge of the reproductive maturation stage is important, as it may determine the husbandry practices to be implemented for a population, particularly during spawning periods.

Disadvantages of indicator

Prompt tracking of reproductive maturation is difficult without recourse to detailed, high cost laboratory testing.

4.9 | Physiological indicators

There is a series of physiological indicators associated with fish welfare, mainly including hormonal, biochemical, osmoregulatory and haematological indices (Table 4.1.).

Table 4.1. Physiological welfare indicators.

	Physiological indicator categories				
			Physicochemical		
	Hormonal	**Biochemical**	**Osmoregulatory**	**Redox**	**Haematological**
Indicators	Cortisol	Glucose	Osmotic Pressure	Muscle pH	Haematocrit
		Lactate		Blood pH	Haemoglobin

Cortisol is considered to be the main stress hormone. Together with the catecholamines adrenalin and noradrenalin it regulates an organism's responses to stress. Unlike catecholamines, which respond immediately, in the order of seconds to a few minutes after stress, cortisol is slower acting but longer lasting. In particular, maximum blood cortisol concentrations in European sea bass are seen one hour after stress, recovering to initial levels in 2 hours, while in gilthead bream maximum levels are seen over the interval 0.5-2 hours, recovering 4 hours later (Fanouraki *et al*., 2011). In addition, since cortisol also regulates other responses such as energy redistribution and osmoregulation, it is regarded as a highly reliable indicator of acute stress (Ellis *et al*., 2012). On the other hand, it is not considered a reliable indicator of chronic stress, as it appears to remain stable under protracted poor living conditions (Tort *et al*., 1996; Rotllant *et al*., 2000b; Barton *et al*., 2005; Di Marco *et al*., 2008; Lupatsch *et al*., 2010; Santos *et al*., 2010; Samaras *et al*., 2018b), but shows an inability to respond naturally following an additional acute stress event (Di Marco *et al*., 2008; Lupatsch *et al*., 2010; Santos *et al*., 2010; Samaras *et al*., 2018b). Cortisol may also be affected by environmental parameters, e.g. it is positively correlated with temperature (Pascoli *et al*., 2011; Samaras *et al*., 2018a): such changes occur as processes whereby the organism prepares to respond to the increased energy demands made by high temperature, and are not necessarily directly associated with poor living conditions (Samaras *et al*., 2018a).

Increase in catecholamine and cortisol concentration leads to elevated concentrations of glucose via gluconeogenesis (i.e. glucose production from non-carbohydrate compounds) and glycogenolysis (i.e. the breakdown of glycogen), respectively (Mommsen *et al*., 1999; Barton, 2002). Glucose is thus also a reliable indicator of acute stress, showing slower and longer-lasting response to stress than cortisol (Fanouraki *et al*., 2011; Samaras *et al*., 2016, 2018a). More specifically, maximum blood glucose concentrations in European sea bass are observed 1-2 hours after stress, with recovery to initial levels in 24 hours, while in gilthead sea bream highest response is seen in 2 hours, and recovery in 8 hours (Fanouraki *et al*., 2011). On the other hand, blood glucose concentrations are affected by nutritional state, since they show elevated levels for 3-6 hours after a meal in European sea bass, and 1-3 hours in gilthead sea bream, with recovery to previous levels after 12 hours in both species (Peres and Oliva-Teles, 1999b; Peres *et al*., 1999; Robaina *et al*., 1999). Although generally speaking both species are considered capable of maintaining glucose concentrations within a narrow range via homeostatic mechanisms, e.g. by storing excess carbohydrates in glycogen, the qualitative composition of feed may also bring about minor changes in glucose (Enes *et al*., 2011). Lastly, in correctly assessing the indicator it should be borne in mind that blood glucose concentrations are affected by environmental parameters such as temperature (Samaras *et al*., 2016, 2018a; Papaharisis *et al*., 2019).

Lactate is also an acute stress indicator, particularly as regards that involving intense muscle activity. Lactate is the end product of anaerobic metabolism, which occurs when activity is intense and continuous, and needs cannot be catered for by aerobic metabolism. After acute stress, lactate concentration rapidly increases as early as 30 minutes after stress, recovering to normal levels in 4 hours (Fanouraki *et al*., 2011). It thus constitutes a reliable indicator for operations that provoke muscle activity (e.g. increased swimming activity and/or acceleration). Furthermore, increases in blood lactate concentration may be observed in situations where oxygen saturation is low (Magnoni *et al*., 2017; Martos-Sitcha *et al*., 2017; Cadiz *et al*., 2018a), as meeting energy demands via aerobic metabolism is more difficult.

Lactate accumulation in muscle leads to it being released into the blood, resulting in a drop in both muscle and blood pH (Milligan, 1996; Samaras *et al*., 2016). For that reason, lactate concentration both in blood and in muscle pH is often used to assess the effect of harvesting methods on the stress caused to fish, time to onset of rigor mortis, flesh quality and on product preservation time (Trocino *et al*., 2005; Bagni *et al*., 2007; Matos *et al*., 2010; Papaharisis *et al*., 2019).

The osmotic pressure of serum or plasma is also regarded as a highly reliable indicator of acute stress, as well as of an individual's overall physiological condition. In a hypertonic environment such as sea water (approx. 1.000 mOsm kg^{-1}) fish must actively maintain their osmotic balance (320-380 mOsm kg^{-1} for both species studied here) by consuming energy. Organs such as the skin, kidneys, gut and especially the gills participate in maintaining this balance, while cortisol is one important hormone contributing to it (Mommsen *et al*., 1999). Any sudden change in water salinity causes serious problems for osmotic regulation and can lead to death (Cataudella *et al*., 1991; Marino *et al*., 1994; Mabrouk and Nour, 2011). A fish's physiological state may affect its capacity to regulate osmotic pressure, e.g. when fasting, which leads to osmoregulatory inability following changes in salinity (Sinha *et al*., 2015).

Haematocrit and haemoglobin are haematological indicators that reflect the blood's oxygen carrying capacity. The haematocrit describes the volume taken up by red blood cells as a percentage of total blood volume. Defining the ranges of normal haematocrit and haemoglobin levels is difficult, since beyond the individual's physiological state they are also affected by environmental parameters such as temperature, photoperiod and oxygen (Lupi *et al*., 2005; Pascoli *et al*., 2011; Samaras *et al*., 2016; Fazio *et al*., 2018), as well as stress (Fazio *et al*., 2015; Samaras *et al*., 2016). Generally speaking, scientific literature reports haematocrit levels of between 30-50% for healthy, unstressed individuals of both species (Pavlidis *et al*., 1997; Caruso *et al*., 2005; Lupi *et al*., 2005; Pascoli *et al*., 2011; Fazio *et al*., 2015, 2018; Samaras *et al*., 2016).

Measurement method

Assessing the concentrations of cortisol, glucose, lactate, osmotic pressure, haematocrit and haemoglobin requires blood sampling. Glucose, lactate, haematocrit and haemoglobin measurements can be carried out on total blood using portable measuring devices, following careful evaluation, and hence are not considered to be exclusively laboratory-based indicators. On the other hand, measuring cortisol and osmotic pressure calls for specialist equipment and trained staff, and are laboratory-based indicators. Measuring muscle pH involves killing fish and taking readings using a pH meter with special sensors for measuring the pH of flesh. With the appropriate equipment it can be assessed outside a laboratory.

Advantages of indicator

Cortisol, glucose, lactate and osmotic pressure indicators accurately depict an organism's response to acute stress situations. On the other hand, haematological indicators are highly useful in assessing an individual's physiological state and health. Furthermore, biochemical and haematological indicators are inexpensive and can be measured outside a laboratory, even at floating pens in the sea.

Disadvantages of indicator

Almost all of the abovementioned indicators are affected by environmental parameters. Defining normal ranges is thus difficult, making it necessary to compare concentrations before and after operations. Cortisol and osmotic pressure are more costly to measure (equipment outlay in the case of osmotic pressure) and require laboratory analysis by specially trained staff. Measuring muscle pH involves killing animals.

4.10 | Behaviour

Behaviour is hard to measure as an indicator, but one of especial importance in some operations. Thus, when fish are stressed by an external stimulus they are likely to display "panic behaviour", which is mainly observed in European sea bass. In such cases the fish make vigorous, rapid movements in indeterminate directions, and may attempt to exit the water. Injuries are common in this context, as fish may collide with each other or with cage nets or tank walls. It is important to record behaviours of this type when carrying out operations such as crowding, netting and transport.

Apart from the above, displays of aggression and competition are one further behaviour that may reflect poor welfare conditions. In the two species under study, behaviours of this type most often occur during feeding; the competition that then arises may be indirect, due to some fish reacting faster and more intensely to feed delivery (scramble competition), or directly expressed via aggression (EFSA, 2008; Attia *et al*., 2012). These behaviours are manifested more intensely during periods when feed intake is limited (Goldan *et al*., 2003; Andrew *et al*., 2004; Papadakis *et al*., 2016; Oikonomidou *et al*., 2019), thus indirectly reflecting poor welfare conditions.

Measurement method

This indicator is usually recorded via visual observation. There are currently no measurable assessment systems for “panic behaviour”, which can thus simply be recorded as “present” or “absent”. Aggression and competition are measurable behaviours in laboratory conditions (e.g. number of aggressive behaviour displays per unit of time), but are difficult to quantify in rearing conditions in fish cages.

Advantages of indicator

An important indicator for monitoring the soundness and severity of a husbandry practice.

Disadvantages of indicator

In the case of “panic behaviour”, the indicator can only be recorded as “present” or “absent”. Competition and aggression, on the other hand, are extremely difficult to measure in floating fish cages.

4.11 | Summary

- Biological welfare indicators provide valuable information on the state of fish. They are divided into two broad categories: those examined in the field; and those examined in the laboratory, as they necessitate the use of specialist equipment.
- Different indicators vary from others in terms of measurement method and certain advantages and disadvantages. For that reason, concurrent assessment of more than one indicator is often required to gain a fuller picture of welfare conditions.
- Several of the field indicators (e.g. mortality, feed consumption) are recorded by fish farm staff while carrying out routine procedures and constitute the most direct assessment of fish welfare.
- Certain indicators (e.g. lower than expected appetite for food or rapid respiration rate) may serve as rapid warnings, and should be followed by detailed examination of other, more accurate indicators.
- Wherever the need arises for detailed recording of an operation’s effects on fish welfare, more accurate field indicators such as glucose and lactate blood concentration or even laboratory indicators such as blood cortisol concentration should be used.

5 | Operational and Laboratory-based Welfare Indicators (OBWIs and LABWIs)

The purpose of operational welfare indicators is to provide a simple, reliable and prompt assessment of the welfare conditions of farmed fish. In most cases it is necessary to use more than one indicator to draw safe conclusions on fish condition. By way of illustration, during transport it is vital to monitor a series of environmental indicators such as oxygen saturation, as well as biological ones such as mortality, behaviour and respiration rate.

As regards use, indicators can be subdivided into:

(i) simple operational indicators not involving fish handling
(ii) more specialised operational indicators involving fish handling and
(iii) indicators requiring laboratory equipment (Figure 5.1.).

An additional distinction can be drawn between:

(a) indirect (environmental) indicators, such as oxygen and salinity, and

(b) biological indicators concerning the animals themselves, such as health, behaviour and physiological indicators.An additional distinction can be drawn between:

Furthermore, with regard to availability and implementation capacity, indicators are divided into:

(i) available Operational Indicators,
(ii) available Laboratory-based indicators,
(iii) available but not commonly used indicators and
(iv) indicators in development.

Examples of these indicators are given in Table 5.1., with emphasis on categories (iii) and (iv), as the remaining ones were analysed in detail in chapters 3 and 4. The uncommonly used indicators concern those for which there are no reference limits vis-à-vis the developmental stage, environmental conditions and clinical picture of fish that enable conclusions to be drawn on their welfare condition.

In some cases, assessment of one environmental indicator alone (e.g. low oxygen) may suffice to lead to a decision (give oxygen). Nevertheless, there are cases where further investigation of more detailed indicators (e.g. blood sampling) or even laboratory analysis (cortisol count) is called for in assessing fish condition.

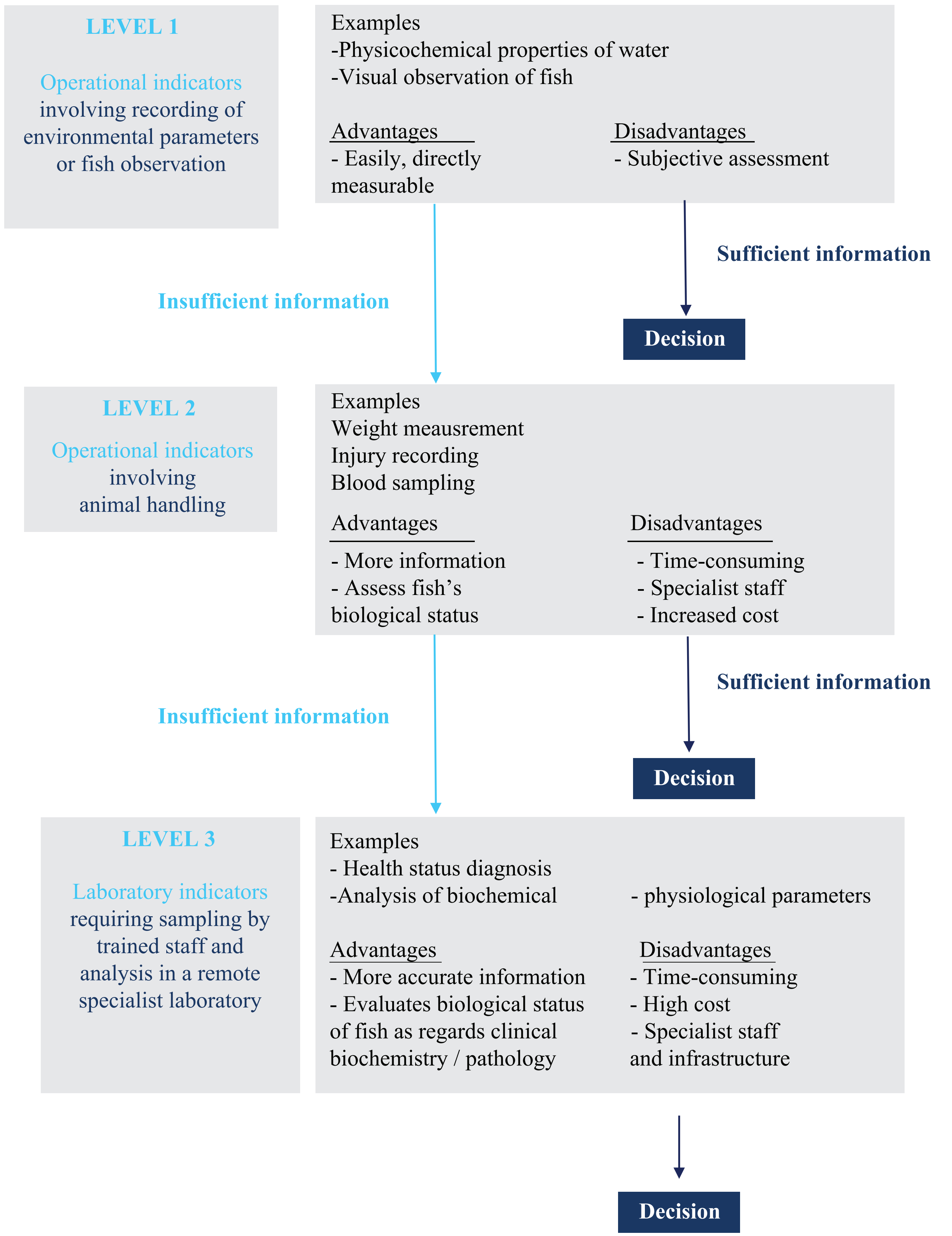


Figure 5.1. How indicators are used in welfare assessment and decision making (Adapted from Noble et al., 2018).

Table 5.1. Summary presentation of indicators by type, availability and implementation capacity.

Available Operational Indicators	Available Laboratory Indicators	Non-used Indicators	Future indicators in development
Temperature	Health	Biochemical profile (cholesterol, proteins etc.)	Precise recording of behaviour (swimming, distribution in space)
Oxygen saturation	Cortisol levels	Electrolyte profile (potassium, sodium, calcium etc.)	Molecular
Salinity	Glucose levels	Liver / kidney function	Genomic
Rearing density	Blood levels		Precision aquaculture (sensors, artificial intelligence etc.)
Mortality	Osmotic pressure		
Growth			
Nutrition			
Injuries			

6 | Detailed presentation of Welfare Indicators by production stage and husbandry practice

6.1 | Assessing Operational Welfare Indicators by production stage

6.1.1 | Brood fish

Brood fish are reared in land-based installations, so that environmental rearing and reproductive conditions can be controlled, and animals can be handled. Brood stock management presents challenges different from fish rearing for production in floating fish cages. The two main peculiarities concern: (i) rearing technology (covered, land-based tanks using open or closed-circuit systems) and (ii) the operations undertaken to identify sex and monitor

Challenges to welfare

Brood fish represent a small percentage of the entire population reared in aquaculture. As they are reared in land-based installations, there are some parameters that may represent a challenge to welfare.

- **Environmental parameters of water.** Rearing water quality is of primary importance for fish welfare. As brood fish are reared in land-based installations with open or (during vitellogenesis/ egg laying periods) closed water circulation systems (very often using boreholes), several of the parameters may change differently and/or more markedly than in the sea. Water temperature, oxygen and pH must thus be monitored and adjusted where necessary to secure optimal conditions.

- **Stocking density.** As in any rearing system, stocking density is an important husbandry parameter in fish wellbeing. In addition, as broodfish are usually large-sized and may show behavioural changes during spawning periods, both density and stocking volume demands differ from those at other stages in the production cycle.

- **Tank operations.** Everyday operations in tanks such as cleaning and routine measurements may impact fish welfare. In particular, reduced appetite for food following tank cleaning has been observed in European sea bass (Rubio *et al*., 2010).

- **Hygiene.** Since rearing water derives from installations outside the farms, usually via boreholes, particular attention must be paid to hygiene. Mechanical and antimicrobial filters should be used for biosecurity and hence stock welfare.

Apart from the above, a number of practices carried out on brood fish may negatively impact fish welfare. These include:

- **Individual fish tagging.** Individual fish tagging only concerns a small percentage of fish used in genetic selection programmes, and so a very low percentage of the total fish used in Greek aquaculture. Thus, any fish requiring individual identification are fitted with PIT (Passive Integrated Transponder) tags. The tagging process involves practices such as crowding, catching, exposure to air and tag implantation, usually hypodermically in the abdominal area. Reading the transponder is also a process involving many of the aforementioned practices.

- **Abdominal massage (stripping) / biopsies to determine sex / maturation stage.** Procedures for determining sex and maturation stage in brood fish include stripping, i.e. abdominal massage to release milt, where present, plus biopsies to identify female individuals and enable detailed laboratory monitoring of maturation stage. Theenvise practices raise issues that must be monitored using welfare indicators, as they include crowding, catching, exposure to air, possibly anaesthesia and, lastly, handling itself.

- **Altering environmental parameters for out-of-season egg production.** Changes in photoperiod and temperature to induce spawning are commonly implemented in breeding stations to produce out-of-season eggs. Although this technique is considered relatively fish-friendly, possible effects on brood stock welfare should be monitored using indicators such as feed consumption, health and behaviour.

- **Hormone therapy.** Fish welfare may be affected when hormone therapies are administered to brood stocks to induce and synchronise spawning. Thus, operations such as crowding, catching, exposure to air, anaesthesia and hormone therapy itself may have marked effects on welfare, rendering them high-risk techniques, particularly for biologically and/or commercially valuable fish (Mylonas *et al*., 2004).

- **Genetic selection.** Genetic selection is carried out using current scientific knowledge and strict selection criteria to avoid potential undesirable crossbreeding. Nevertheless, possible co-selection of undesirable characters may bring about a reduction in fish welfare. In theoretical terms, it has also been reported that combining high genetic capacity for growth, feed consumption and use may prompt an organism to achieve very high performances, which is deemed unacceptable from a welfare point of view. (Hastein, 2004).

- **Change in social relations.** This challenge mainly concerns gilthead sea bream, which is a protandrous hermaphrodite species. Inversion or non-inversion of sex in the species is affected by social parameters, among other things (Zohar *et al*., 1978). Thus, young males entering the stock may alter social balances, leading to an increase in sex inversion in older male individuals (FAO, 2005). Furthermore, the presence of more females delays sex inversion in younger males.

Operational Welfare Indicators in brood fish rearing

Environmental indicators	Biological indicators	
	Stock indicators	Individual indicators
Oxygen	Mortality	Health
Temperature	Appetite	Injuries
Salinity	Growth	Sexual maturation and spawning
pH	Behaviour	
Stocking density		
Lighting		
Turbidity		

Environmental indicators

Oxygen saturation. In land-based tanks oxygen saturation must be monitored continually, as changes in fish activity (e.g. feeding, stress from routine husbandry practices) or water recycling rate may bring about a significant reduction in oxygen. Furthermore, in installations where tanks have an external oxygen supply, possible hyperoxia should be monitored for saturations exceeding 100%.

Temperature. Water temperature in land-based tanks may remain constant year-round, e.g. when water from boreholes is used, or it may vary either in line with environmental standards or in a controlled way for husbandry purposes. At any event temperature must be recorded daily, as sudden changes cause welfare problems. In the case of brood fish in particular, temperature adjustments are usually made to induce vitellogenesis and spawning. When this takes place, changes should be mild – not exceeding a recommended 1°C per day – so as not to cause stress to fish.

Salinity. In land-based tanks supplied by boreholes, it is common for salinity to be lower than in the sea. Although this is not anticipated to pose problems for the welfare of European sea bass or gilthead sea bream, water must be monitored for potentially marked changes in salinity. Fluctuations of this type negatively impact fish welfare and, in extreme instances, may even lead to mortality. In that context, especial attention should be paid during the transportation of brood fish from the sea to land-based installations, such as when adding new individuals to the breeding nucleus of selection programmes, so they are not subjected to acute and marked changes in salinity.

pH. According to the EFSA report (2008), water pH should be lower than 6.5 or higher than 8.5. As with salinity, it is important to avoid sudden changes in pH level, which may be seen at tank outflow points. For that reason, in common with oxygen, pH should be measured at tank water inflow and outflow points.

Stocking density. Stocking density is a rearing parameter determined by producers. High stocking densities may lead to reduced welfare due to various factors, whether environmental (e.g. reduced oxygen), biological (e.g. injuries) or even social (e.g. uneven feed distribution, limitation of natural behaviours). Particularly as concerns brood fish, which are usually large-sized animals, care should be taken not only over stocking density, but also over available rearing volume, so as to guarantee the free expression of swimming and social behaviours.

Lighting. Lighting (the intensity, quality and variation in the duration of light) is an important environmental parameter during stock fish rearing, as it is manipulated to produce out-of-season eggs. The effects of changes in photoperiod should be monitored together with other supplementary biological welfare indicators such as feeding appetite, growth and behaviour. As regards lighting quality, in other words intensity and wavelength, ideal conditions for brood stock rearing in tanks remain unclear.

Turbidity. Water turbidity refers to the presence of suspended, colloidal or dissolved matter. It may cause or be an indication of problems in other parameters, such as reduced oxygen or elevated bacterial density in water. It should be monitored accordingly and, when deemed necessary, further environmental indicators should be examined to pinpoint the problem.

Biological indicators

Mortality. Mortality is potentially the final stage of poor living conditions. It should be recorded daily, with supplementary indicators being monitored whenever unanticipated increases in mortality rate are observed, so as to determine the cause.

Appetite. This is a reliable indicator in promptly establishing that living conditions have deteriorated. Particularly in rearing conditions where environmental conditions such as temperature and oxygen saturation are controlled and stable, unanticipated loss of appetite can serve as a warning for thorough monitoring of additional indicators. It has the advantage of not requiring animal handling and sampling.

Growth. Unanticipated reduction in body growth is also an indication of poor living conditions worthy of further investigation, particularly as regards health. Of course, assessing growth requires repeated weight sampling, which in itself has a negative effect on welfare and is usually avoided in broodfish stocks.

Behaviour. The manifestation of normal swimming behaviour should be monitored, and any aberrations such as "panic behaviours" should be recorded. Furthermore, in good living conditions there should be no displays of competition and aggression between individuals. The emergence of such behaviours must thus be recorded, and its causes investigated using other indicators.

Health. Fish health should be monitored by sampling on a regular basis, so as to avert situations leading to mortality. Some checks such as alterations in appearance, exophthalmia and macroscopic monitoring of gill condition can be carried out by farm staff, whereas more detailed analyses demand detailed checking by registered veterinary staff. Prompt diagnosis of health problems can contribute to their rapid, appropriate resolution, thus averting effects on fish welfare.

Injuries. Injuries to fish raised in land-based installations can occur due to collisions either with tank walls or between fish, e.g. during crowding. In particular, injuries can be recorded indirectly in the course of such operations, by observing the presence or absence of blood or scales in the water. More detailed monitoring requires the fish to be examined individually for injuries to the skull, skin or fins.

Reproductive maturation and spawning. Since brood fish constitute a fish farm's breeding nucleus, their reproductive state should be monitored. This is achieved by invasive methods such as stripping, biopsies or ultrasound, the severity of which should likewise be monitored using biological indicators.

6.1.2 | Pre-growing

Pre-growing is the stage in the production cycle lasting from the end of larval rearing until fish are transported to floating fish cages in the sea. During this stage fish are reared in open-circuit tanks at land-based installations. The object of this period is to acclimatise fish to commercial feed and increase their body weight, so as to prepare them for transfer and rearing in mariculture cages.

Welfare challenges

As pre-growing takes place in open-circuit land-based installations, a number of parameters may present a challenge to welfare. These include:

- **Environmental parameters of water.** Water quality is of primary importance to fish wellbeing. As rearing occurs in tanks, many of the environmental parameters such as temperature, oxygen and lighting can be regulated and determined by producers. Others, such as salinity and pH, are characteristics of the source water (e.g. sea or borehole), and hence must be monitored and adjusted where necessary to secure optimal conditions.

- **Stocking density.** As in any rearing system, density is an important husbandry parameter for fish wellbeing. High stocking densities may both negatively impact fish growth and provoke injuries.
- **Tank operations.** Everyday operations in tanks such as cleaning, feeding and routine measurement may impact fish welfare.
- **Fish operations.** Various operations are carried out during the pre-growing stage, including transfer from the hatcheries where larval rearing takes place to pre-growing installations, grading to ensure uniform size distribution, and veterinary procedures such as vaccination. These operations in themselves are practices thoroughly analysed in terms of their effects on welfare below (Chapter 6.2.).
- **Health.** Since rearing water derives from installations outside the farms, usually via boreholes, particular attention must be paid to hygiene. Mechanical and antimicrobial filters should be used for biosecurity and hence stock welfare.

Operational Welfare Indicators during pre-growing

Environmental indicators	Biological indicators	
	Stock indicators	Individual indicators
Oxygen	Mortality	Health
Temperature	Appetite	Injuries
Salinity	Growth	Respiration rate
pH	Behaviour	
Stocking density		
Lighting		
Turbidity		

Environmental indicators

Oxygen saturation. Oxygen should be monitored constantly and adjusted where necessary so as to remain at optimal rearing levels. Changes in fish activity (e.g. feeding, stress from routine husbandry practices) or water recycling rate may lead to a significant drop in oxygen. Furthermore, in installations where tanks have an external oxygen supply, possible hyperoxia should be monitored for saturations exceeding 100%.

Temperature. Temperature is a significant factor in fish growth rate. Water temperature in land-based tanks may remain constant year-round, e.g. when water from boreholes is used, or it may vary either in line with environmental standards or in a monitored way for husbandry purposes. Both species studied here have wide temperature tolerance ranges, so a more important role is played by the avoidance of sudden temperature changes, which cause welfare problems. Especial attention should thus be paid to possible water temperature differences between the hatchery and pre-growing when fish are being transferred. Temperature should also be monitored constantly throughout rearing for potential unforeseen changes.

Salinity. When water from boreholes is used, it is common for salinity to be lower than that in the sea. This is not anticipated to present a problem for the welfare and vigour of European sea bass and gilthead sea bream during pre-growing (Laiz-Carrión *et al.*, 2005); there are even reports of better growth in low salinities at this stage (Eroldoğan and Kumlu, 2002). As in the case of temperature, sudden changes in water salinity are to be avoided, as in extreme cases it may lead to mortality.

pH. According to the EFSA report (2008), water pH should be no lower than 6.5 and no higher than 8.5. pH is one characteristic of water that is not anticipated to change markedly in open-circuit systems. Nevertheless, in properly monitoring water quality pH should be recorded at tank water inflow and outflow points.

Stocking density. Stocking density is a rearing parameter determined by producers High stocking densities are anticipated to negatively impact fish growth, and may lead to reduced welfare due to various factors, whether environmental (e.g. reduced oxygen), biological (e.g. injuries) or even social (e.g. uneven feed distribution, limitation of natural behaviours).

Lighting. In instances where artificial light is used during pre-growing, its intensity, quality (spectrum) and photoperiod may affect fish welfare, though ideal conditions remain unclear. These features are usually peculiar to the infrastructure at installations, and are not anticipated to vary during rearing.

Turbidity. Water turbidity refers to the presence of suspended, colloidal or dissolved matter. It may cause or be an indication of problems in other parameters, such as reduced oxygen or elevated bacterial density in water. It should be monitored accordingly and, when deemed necessary, further environmental indicators should be examined to pinpoint the problem.

Biological indicators

Mortality. Increased mortality is an indicator of poor living conditions. It should be recorded daily during feeding, as well as after operations such as transport, sorting etc. Any unanticipated increase in mortality rate should act as a warning sign for the monitoring of supplementary indicators, so as to establish the cause.

Appetite. Reduced appetite is a potential indicator that living conditions have deteriorated. Where environmental and husbandry conditions remain stable, reduced feeding appetite is warning that other indicators such as health should be monitored. As an indicator, it has the advantage of not requiring animal handling or sampling.

Growth. Lower than anticipated body growth is also an indicator of poor living conditions meriting further investigation, especially as regards health disorders in fry. Of course, assessing growth requires repeated weight sampling, which negatively impacts welfare.

Behaviour. Deviations from natural swimming behaviour should be monitored. Under normal conditions there should be no observable competition or aggression between individuals, particularly after sorting to normalise size distribution. Any appearance of behavioural deviations should be recorded, and causes monitored using other indicators.

Health. Fish health should be monitored by sampling on a regular basis to avert situations causing mortality. Furthermore, various veterinary procedures such as vaccinations are carried out in the pre-growing stage, the effects of which should be monitored using other indicators.

Injuries. Injuries can occur to fish during pre-growing either due to collisions with tank walls during rearing, or husbandry practices such as transport and sorting. In particular, injuries can be recorded indirectly in the course of such operations, by observing the presence or absence of blood or scales in the water. More detailed monitoring can be carried out by examining fish individually for injuries to the skull, skin or fins.

Respiration rate. Respiration rate can be used as a non-specialised, non-invasive stress indicator when carrying out stress-inducing husbandry practices. In particular, increase in respiration rate may result from stress, which should be recorded for retrospective assessment of handling.

6.1.3 | On-growing

In Greek aquaculture, almost all European sea bass and gilthead sea bream production is carried out in floating pens in the sea. Fish spend the greater part of their life in this environment up until harvesting and killing. It is an environment typified by the stability of certain factors such as salinity and pH, as they are characteristics of the sea that do not vary markedly but also present many challenges, such as seasonal changes in temperature and oxygen, infections and parasite infestations, as well as stress-inducing operations.

Welfare challenges

- **Environmental parameters of water. W**hen rearing fish in floating cages in the sea, water quality is determined by environmental conditions. Salinity and pH are relatively stable in most cases, whereas parameters such as temperature and oxygen show seasonal variation. Oxygen in particular may be a limiting factor on growth and fish welfare in the summer months, even leading to the emergence of mortality.
- **Stocking density.** As in any rearing system, density is an important husbandry parameter for fish wellbeing. High stocking densities may negatively affect fish growth and the emergence of injuries and illnesses. Apart from stocking density, especially in cages where thousands of fish are reared together, “available space” or the rearing volume available for fish to display natural swimming and social behaviours is of particular importance for their welfare.
- **Cage operations.** A series of operations carried out in cages may negatively affect welfare, such as net changes, diving work or cage transportation.
- **Fish operations.** Various operations are carried out on fish during the on-growing stage in cages, such as crowding for handling or sampling, transport, treatments and, finally, harvesting and killing. All of these operations may have a negative impact on fish welfare and should be monitored using both indirect and biological indicators. Assessment methods for these practices are further analysed in a subsequent chapter (Chapter 6.2.).
- **Hygiene.** The marine environment teems with organisms of potential harm to fish. In intensive rearing conditions with thousands of fish living together in a small volume, disease and parasite outbreaks may occur. It is thus necessary to take preventive measures such as vaccination and regular health monitoring for prompt disease diagnosis. Maintaining hygienic conditions is likewise extremely important to prevent external pathogens spreading to farmed populations.

Operational Welfare Indicators during on-growing

Environmental indicators	Biological indicators	
	Stock indicators	Individual indicators
Oxygen	Mortality	Health
Temperature	Appetite	Injuries
Salinity	Growth	Respiration rate
pH	Behaviour	Haematocrit and haemoglobin levels in blood
Stocking density		Glucose and lactate levels in blood
Lighting		Cortisol level in blood (laboratory indicator)
Turbidity		

Environmental indicators

Oxygen saturation. Oxygen saturation is only likely to be low in certain situations, as the sea in areas where aquaculture farms are located is not expected to be hypoxic. Thus situations such as diameter reduction or total occlusion of cage mesh due to improper cleaning, high stocking densities, high temperature leading to a reduction in the oxygen-bearing capacity of water or a combination of all of the above will result in a drop in oxygen saturation. In extreme cases over the summer months, this may even lead to the emergence of mortality. Oxygen should thus be monitored systematically, and all preventive measures taken to avert deficit situations.

Temperature. Water temperature is a markedly seasonal environmental parameter. It greatly affects fish growth, activity, behaviour and physiology. Producers have no control over this parameter in cages, so constant recording and monitoring is required in properly managing fish needs. It is important for measurements to be taken from different depths in cages, as temperature may vary with depth.

Salinity. Salinity is not anticipated to have a significant impact on fish welfare during on-growing in the sea, as it generally stable, with slight variations mainly due to heavy rainfall.

pH. Like salinity, pH rarely causes problems for fish welfare, as it is not anticipated to vary significantly, except in cases of poor water circulation due to improper net cleaning.

Stocking density. Stocking density is determined by producers and may affect several rearing factors. In particular, high densities are anticipated to negatively affect fish growth, and may lead to problems in water quality or the manifestation of natural behaviours in fish, as well leading to as injuries. Another important parameter of fish cages is the volume available to fish, as increase in available volume has been seen to lead to better welfare conditions (Samaras *et al.*, 2017).

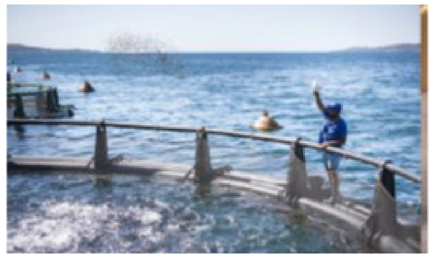
Lighting. The light at fish cages is almost always natural. In such cases, the production process is not anticipated to have any impact on welfare. Earlier experimental techniques—which never gained widespread acceptance and are no longer in use—implemented changes to photoperiod via artificial lighting, so as to prevent early reproductive maturation in European sea bass. In such cases involving the use of artificial lighting, it is vital to assess its impact on welfare using other supplementary indicators.

Turbidity. Water turbidity refers to the presence of suspended, colloidal or dissolved matter. It is rare at sea, but in instances where increased turbidity is observed, fish welfare must be monitored in detail using other indicators.

Biological indicators

Mortality. As in previous rearing types, any increase in mortality rates is a sign of poor living conditions. Of course, given that there are no published mortality curves for the species under study, it is important for comparative analysis to be carried out at every farm on the basis of its own mortality history.

Appetite. Systematic monitoring of feed consumed by fish in order to estimate appetite is difficult to perform in cages. Feeding is usually carried out using feeding tables, which determine daily consumption on the basis of environmental conditions, fish size and the qualitative composition of feed. In such cases, reduced feeding can be evaluated indirectly from growth.



Growth. In a healthy fish, feed consumption is expected to lead to increased body weight. When growth is lower than anticipated there may be some problem in fish living conditions, leading either to non-consumption of all the feed provided or to unsatisfactory conversion efficiency. At all events, problems in fish growth should be thoroughly examined using additional indicators, so as to assess what is causing them.

Behaviour. Social relations are not expected to be strongly established during rearing in fish cages, so stable hierarchies do not emerge. This reduces the likelihood of direct competition and aggression, particularly during feeding. Nevertheless, indirect competition over food does exist, as some fish are faster than others at reaching feed, where sporadic instances of aggression may also arise. One further behaviour that may reflect poor living conditions is swimming. Thus, continuous, systematic display of unnatural swimming behaviour such as "panic swimming", manifested in sudden movements and marked changes of direction, shoal fragmentation and the avoidance of swimming in particular areas of the cage are indicative of stress, and hence poor living conditions.

Health. Fish health must be monitored by sampling on a regular basis, so as to avert situations leading to mortality. Veterinary procedures such as macroscopic examination, vaccination and treatments are frequent in this production stage; their impact on welfare should be monitored using other indicators.

Injuries. In the course of rearing in cages, injuries may occur to fish either due to collision with nets during rearing or to various other husbandry practices, such as transportation, weight and health sampling etc. In particular, injuries can be recorded indirectly in the course of such operations by observing the presence or absence of blood and/or scales in the water. More detailed monitoring requires the fish to be examined individually for injuries to the skull, skin or fins.

Respiration rate. Respiration rate can be used as a non-specialised and non-invasive stress indicator when carrying out stress-inducing husbandry practices. In particular, increased respiration rate may result from stress, and should be recorded for the retrospective assessment of operations.

Blood haematocrit and haemoglobin levels. Although these indicators require: a) blood sampling from fish, and thus a series of stress-inducing operations, such as netting, exposure to air and anaesthesia and b) reference values, they are useful in assessing fish vigour. Low haematocrit and haemoglobin levels may reflect problems in fish living conditions or health.

Blood glucose and lactate levels. These indicators can be used to assess the severity of husbandry practices. For instances, taking samples before a practice such as crowding can assist in assessing the stress caused, in terms of both the intensity and the duration of the response. Lactate in particular is an indicator associated with muscle activity and is thus useful in instances where operations are anticipated to trigger increased activity in fishes. Use of these indicators will be analysed in greater depth in the chapter on evaluating individual practices (Chapter 6.2.).

Blood cortisol level. Cortisol is one of the most important hormones involved in regulating stress. Thus, in cases where it is necessary to monitor the stress caused by an operation, cortisol is a reliable indicator. Analysing it requires special laboratory equipment and trained staff, thus rendering it a laboratory indicator. A more analytical presentation of its use is given in the description of assessing individual practices (Chapter 6.2.).

6.2 | Assessing Operational Welfare Indicators by husbandry practice

6.2.1 | Crowding

Crowding is a very frequent practice when managing fish, both in cages at sea and in tanks at land-based installations. It is a term used to describe a marked, abrupt increase in rearing volume, resulting in large numbers of fish being in a given unit of volume. This practice is combined with and usually precedes other practices such as transport, sorting, vaccination and killing. The need for it is thus considerable, even though it does pose certain challenges to welfare, often in combination with practices aimed at improving welfare, such as vaccination.

In cages, crowding is achieved either by lifting nets completely or by trapping some of the fish in smaller areas formed by partial lifting. In tanks it is usually carried out by draining, with or without cutting off the inflow water.

Welfare challenges

- **Reduction in available oxygen.** On account of the increased stocking volume and/or reduced volume accompanying crowding, there is a risk that the oxygen available to fish will drop. Furthermore, as crowding is a stressful procedure for fish, there is an observable increase in oxygen demands to cover metabolic needs, further amplifying the risk arising from the drop in oxygen.
- **Swimming and behaviour.** The swimming behaviour of fish is hindered under crowding conditions due to the loss of living space. Contact and fish striking each other as a result of crowding may lead to scale loss and/or injuries to fins and skin.
- **Stress.** Abrupt changes in daily stock care and the volume of water available to fish, plus reduction in oxygen or prospective injuries are negative stimuli for the animals. Particularly when abrupt, crowding is one of the main stress factors for both European sea bass and gilthead sea bream. As a typical example, elevated concentrations of cortisol, glucose and lactate in the blood (Rotllant *et al*., 2001, 2003; Guardiola *et al*., 2016) and reduced muscle pH levels (Bagni *et al*., 2007) have been observed in both species following abrupt, intense crowding.

Ways of reducing the negative effects of crowding

- One sound practice widely implemented in Greek aquaculture is to avoid crowding the entire population when not necessary, but to crowd a random subsection sufficient to cater for the reason why the practice in question is being implemented. Determining the needs of the practice from the outset is thus important in assessing the size of population to be subjected to crowding.
- Minimise the duration and intensity of crowding.
- Particularly European sea bass, which is a species sensitive to operations, especial care is required when crowding begins, to avert the emergence of "panic behaviours" that result in injuries to fish
- As regards reduction in oxygen, where observed, additional oxygen can be supplied during crowding.
- Particularly in fish cages, creating very shallow pockets where fish can be exposed to air or become trapped is to be avoided.
- During crowding it is important to monitor the procedure and, where necessary, take the necessary steps to modify it on the basis of operational welfare indicators.

Operational Welfare Indicators during crowding

Environmental indicators	Biological indicators	
	Stock indicators	**Individual indicators**
Oxygen	Mortality	Injuries
Temperature	Behaviour*	Respiration rate
	Injuries	Blood glucose and lactate levels
		Blood cortisol levels (laboratory indicator)

*Such as swimming behaviour, or dorsal fin seen above water.

Environmental indicators

Oxygen saturation. As oxygen saturation may drop dramatically under crowding conditions, levels must be monitored throughout the procedure. As a general recommendation, the EFSA report (2008) mentions that oxygen saturation in rearing conditions should not drop below 40%.

Temperature. The temperature at which crowding occurs is important, as fish metabolism, oxygen consumption (Claireaux and Lagardère, 1999) and even reference levels for physiological stress indicators (Samaras *et al*., 2018a) depend on it, but also because temperature directly affects oxygen saturation in water. Particularly in periods when temperature is high, oxygen saturation is low and the energy demands of fish are higher, thus rendering the drop in oxygen even more harmful to them.

Biological indicators

Mortality. Crowding is not a procedure anticipated to cause mortality. Nevertheless, the number of deaths should be recorded where they may occur, both during the procedure and over an interval of at least 3 days thereafter, so as to pinpoint possible problems in retrospect.

Behaviour. This refers to the observation of behaviours such as burst swimming or exposing the dorsal fin above water. Such behaviours indicate stress, and their appearance is to be avoided.

Injuries. Crowding may cause injuries due to fish striking each other and or cage nets and tank walls, which is why the presence of scales, mucus or blood in the water is one indicator of reduced welfare. Such injuries may not prove fatal during the procedure itself but are lesions that can lead to osmoregulation problems as well as infections. Individual examination of the fish's external condition is a more specific welfare indicator. The commonest injuries occur to the skin, such as loss of scales, and to fins, though there may also be damage to the eyes, the snout and the opercular bone.

Respiration rate. Respiration rate increases both during intense activity and in stress situations. Using this indicator initially necessitates assessing respiration rate before crowding, so as to calculate the difference, as the rate may be affected by external factors such as temperature and oxygen saturation. A further difficulty lies in the fact that measuring respiration rate requires the animal to be stationary or slow moving.

Blood glucose and lactate levels. These two biochemical indicators reach high levels when an animal is under conditions of stress. Lactate in particular is heavily affected by stress, which prompts vigorous exercise (movement) in the animal. During crowding, when intense swimming activities are seen, both indicators are anticipated to increase. Measuring them does not require special laboratory equipment, as they can be gauged with portable instruments. Glucose and lactate show increases in both European sea bass and gilthead sea bream from half an hour after stress, though glucose peaks at between 2-4 hours and lactate at 1-2 hours (Fanouraki *et al*., 2011). Evaluating these indicators more properly also involves taking samples before crowding begins, to serve as base levels, as both glucose and lactate concentrations are affected by season, nutrition, physiological condition and the animal's developmental stage.

Blood cortisol levels (laboratory indicator). Cortisol is one of the most reliable indicators of acute stress. It has emerged that crowding causes a marked increase in cortisol in fish; in European sea bass, this increase is seen as early as 6 minutes from crowding (Rotllant *et al*., 2003). Furthermore, this indicator can be used to assess the duration of stress as reflected in the time taken for values to return to pre-stress levels. Assessing the indicator requires laboratory equipment and specialized staff.

6.2.2 | Transportation

Transporting live fish is a common practice, particularly when moving juvenile fish from nurseries to floating rearing pens. During transportation fish are exposed to a series of stress-inducing practices such as crowding, both when being caught and, in some cases, when being transferred, caught and loaded onto transport facilities, during transportation itself and, lastly, during unloading.

Welfare challenges

- **Operations during the procedure:** Apart from causing stress to fish, crowding and fish loading and unloading operations may also cause injuries and any health problems that result from them.
- **Water quality:** Water quality may deteriorate during transport, with an observable drop in several environmental welfare indicators. Additionally, in periods when rearing water temperature and air temperature differ significantly, there may be marked changes that negatively impact welfare.

Ways of reducing the negative effects of transportation

- Sound practices should be implemented during operations prior to transportation. New methods, such as pumping fish for loading onto transport facilities, can be adopted.
- Deterioration in water quality during transportation should be dealt with. This involves monitoring quality indicators and implementing steps such as adding oxygen, changing water etc. to avert any deterioration in it.

Operational Welfare Indicators during transportation

Environmental indicators	Biological indicators	
	Stock indicators	**Individual indicators**
Oxygen	Mortality	Injuries
Temperature	Behaviour	Respiration rate
Stocking Density	Injuries	Blood glucose and lactate levels
pH		Blood cortisol levels (laboratory indicator)

Environmental indicators

Oxygen saturation. As water is not renewed under transportation conditions, oxygen saturation may decrease dramatically. Oxygen levels should thus be monitored during transport and oxygen added to the water where deemed necessary.

Temperature. Rearing water temperature may change significantly during transportation, especially when it differs markedly from that of the air. Changes in temperature may cause additional stress to fish, leading to elevated oxygen consumption.

Stocking density. Stocking density must be carefully chosen for transport as high densities may lead to more marked deterioration in rearing water quality, particularly as regards the three aforementioned indicators.

pH: Water pH may decrease during transport procedures, due to fish metabolism and the lack of water renewal.

Biological indicators

Mortality. Transportation is a process that may lead to mortality, as it comprises various distinct stress-inducing operations. Any mortalities both during transportation and for some days thereafter should therefore be recorded, so as to pinpoint possible problems in retrospect.

Behaviours. Behaviour such as swimming and shoal formation should be monitored both during the procedure and for some days thereafter, so as to assess the degree of stress inflicted. Recording the time required for feeding to resume once the procedure is completed is another significant parameter.

Injuries. Both the transportation procedure in itself and loading and unloading in particular may cause injuries due to fish striking each other and or cage nets and tank walls, which is why the presence of scales, mucus or blood in the water is one indicator of reduced welfare. Such injuries may not prove fatal during the procedure itself, but are lesions that can lead to osmoregulation problems as well as infections. Individual examination of the fish's external condition is a more in-depth welfare indicator. The commonest injuries occur to the skin, such as loss of scales, and to fins, though there may also be damage to the eyes, the snout and the opercular bone.

Respiration rate. Respiration rate increases both during intense activity and in stress situations. Using this indicator initially necessitates assessing respiration rate before the procedure, so as to calculate the difference, as the rate may be affected by external factors such as temperature and oxygen saturation. A further difficulty lies in the fact that measuring respiration rate requires the animal to be stationary or slow moving.

Blood glucose and lactate levels. These two biochemical indicators reach high levels when an animal is under conditions of stress. Lactate in particular is heavily affected by stress, which prompts vigorous exercise in the animal. Both indicators are expected to increase during transportation. Measuring them does not require special laboratory equipment, as they can be gauged with portable instruments. Glucose and lactate appear to remain elevated in both European sea bass and gilthead sea bream during the course of exposure to new tanks following stress, and up to 4-8 hours thereafter (Samaras *et al.*, 2016, 2018a; Jerez-Cepa *et al.*, 2019). Assessing these indicators more properly also involves taking samples before the procedure begins, to serve as base levels, since both glucose and lactate concentrations are affected by season, nutrition, physiological condition and the animal's developmental stage.

Blood cortisol levels (laboratory indicator). Cortisol is one of the most reliable indicators of acute stress. The entire transportation process includes practices such as crowding and netting as well as a new environment, etc., which have been shown to cause a marked increase in cortisol in fish (Fanouraki *et al.*, 2011; Samaras *et al.*, 2016, 2018b). Cortisol recovers to pre-stress levels faster than glucose and lactate (Fanouraki *et al.*, 2011; Jerez-Cepa *et al.*, 2019). Assessing the indicator requires laboratory equipment and specialised staff.

6.2.3 | Sorting

Size sorting is a procedure carried out at several stages in the production cycle, so as to create groups of uniform size. Sorting is often done in the pre-growing stage, so that the fish can be separated into similarly sized groups, mainly to improve living conditions for smaller-sized fish, though also for better feed delivery. Sorting may also occur during vaccination and the provision of veterinary services, as well as during harvesting, particularly with electrostunning devices. As a procedure it involves various operations such as crowding, catching, exposure to air during sorting, as well as transfer to the new rearing medium. Attention must thus be paid to the distinct practices comprising it.

Welfare challenges

- Operations during the procedure: The operations carried out during the procedure such as crowding, catching and exposure to air cause stress in fish, and may additionally lead to injuries and potential health problems.
- Sorting process: Although size sorting devices are constructed to prevent injury, incorrect use of them may lead to injuries, mainly to the skin, skull and fins.

Ways of reducing the negative effects of sorting

- Sound practices should be implemented before, during and after operations.
- The surfaces used for sorting fish must be wet, without protrusions or rough sides that could injure the animals.

Operational Welfare Indicators during sorting

Environmental indicators	Biological indicators	
	Stock indicators	**Individual indicators**
Oxygen	Mortality	Injuries
Temperature	Behaviour	Health
Stocking Density	Injuries	

Environmental indicators

Oxygen saturation. As described in detail in the section on crowding (6.2.1.), oxygen saturation should be monitored in the course of operations on fish.

Temperature. Water temperature should be monitored both where crowding occurs and in the new medium where fish are to be placed, avoiding exposure to marked differences in temperature.

Stocking density. The density created by crowding prior to sorting should be monitored, so as not to reach the point where parts of fish are above water or there is a dramatic reduction in their swimming behaviour.

Biological indicators

Mortality. As in any procedure, low mortality rates may be observed during sorting or thereafter. Records of them should be kept.

Behaviour. Behaviour such as burst swimming or exposing the dorsal fin above water should be observed; time taken for the resumption of feeding after the procedure should be recorded.

Injuries. As mentioned above, although all preventive measures should be taken to avoid injuries, particularly from sorting devices, it is important to record cases where injury indicators such as blood or scales are seen in the water or the sorter. In extreme cases calling for more precise monitoring, fish may be examined individually for possible injuries, mainly to the skin, skull and fins.

Health. If injuries are present, resultant infections may occur. Consequently, any indications of disease that appear over the days immediately following the handling operation should be investigated more thoroughly.

6.2.4 | Sampling to monitor weight and health

Sampling to monitor weight, health or even welfare indicators, such as blood sampling to assess physiology indicators, is a common practice throughout the production cycle, but one that may have significant effects on fish welfare. It includes practices such as crowding, chasing to catch individuals, exposure to air, anaesthesia in some instances, and handling operations depending on the samples being taken. When carried out in the recommended manner, these practices are regarded as being of mild severity, with a low impact on welfare (EC, 2009; Hawkins *et al.*, 2011). All the same, a series of indicators should be monitored throughout these practices.

Welfare challenges

- **Operations during the procedure:** Operations in the course of the procedure such as crowding, catching, exposure to air and in some cases anaesthesia cause stress to fish and may additionally lead to injuries and potential health problems resulting from them.
- **Anaesthesia:** Measuring weight and examining health or other indicators in fish very often means anaesthetising them. If not administered at the correct dosage depending on the fish's developmental stage and body weight, or if it lasts for longer than recommended, this may pose significant welfare issues, even leading to death.
- **Sampling:** Operations such as biopsies and blood tests carried out on fish during sampling may induce stress.

Ways of reducing the negative effects of monitoring weight and health

- Sound practices should be implemented in the course of operations, before, during and after handling.

Operational Welfare Indicators during sampling to monitor weight and health

Environmental indicators	Biological indicators	
		Individual indicators
Oxygen	Mortality	Injuries
Temperature	Behaviour	Respiration Rate
	Injuries	

Environmental indicators

Oxygen saturation. Oxygen saturation should be monitored both during crowding and in the anaesthesia and recovery tanks. Recovery in particular is more difficult when water oxygen saturation is insufficient.

Temperature. Water temperature in anaesthesia and recovery tanks may vary, especially when there are marked differences between air and water temperature, e.g. on hot summer days. Temperature variation is anticipated to lead to changes in oxygen saturation.

Biological indicators

Mortality. Operations to sample zootechnical characteristics are procedures that may in rare instances lead to mortality. It is thus important to record any mortality during procedures and over some days thereafter, so as to pinpoint possible problems in retrospect.

Behaviour. Behaviour such as swimming and shoal formation should be monitored both during the procedure and for some days thereafter, so as to assess the degree of stress inflicted. Recording the time required for feeding to resume once the procedure is completed is another significant parameter.

Injuries. Operations aimed at sampling morphometric characteristics may lead to injuries, as they include practices such as catching and exposure to air. Thus, one indicator of reduced welfare at the stock level is the presence of scales, mucus or blood in the water. Furthermore, when deemed necessary, individual examination of the fish's external condition as a more detailed indicator may be carried out. Of course, this is only recommended in instances where it is deemed necessary, as individually examining fish involves repeating the sampling procedure.

Respiration rate. Respiration rate can be utilized as a procedural stress indicator. Using this indicator initially necessitates assessing respiration rate before the procedure, so as to calculate the difference, as the rate may be affected by external factors such as temperature and oxygen saturation. A further difficulty lies in the fact that measuring respiration rate requires the animal to be stationary or slow moving.

6.2.5 | Administering veterinary treatments

Although operations to administer veterinary treatments or preventive measures aim to safeguard fish health, they may cause stress nonetheless. A number of veterinary procedures such as vaccinations at a particular stage in the production cycle are routine for every fish stock, while others including antibiotic or antiparasitic treatments are implemented when necessary. In any event, implementing veterinary practices is essential, and aims at improving fish living conditions, but welfare indicators should be recorded during implementation.

Welfare challenges

- **Operations during the procedure:** Assessing fish health initially requires sampling, as described in previous sections. Furthermore, implementing veterinary treatments or preventive measures involves animal handling operations. Thus, during vaccination fish are exposed to a series of operations that include crowding, catching, anaesthesia and, lastly, inoculation itself. Similar operations are necessary in the case of treatments, while the treatment procedure itself often involves placing fish in water that contains an antibiotic or antiparasitic chemical agent.
- **Effects of veterinary procedures:** Implementing preventive measures or treatment may in itself represent a challenge to welfare. In particular, implementing practices such as transferring fish to low salinity water or administering chemical antiparasitics to combat pathogens (Athanasopoulou, 2001) is anticipated to impact fish welfare. Furthermore, particularly when injected, vaccinations may lead to side effects such as reduced growth rate, chronic peritonitis, fibrous adhesions and granulomas in the peritoneal cavity (Papadopoulos *et al*., 2008).
- **Water quality when carrying out veterinary procedures:** Implementing preventive measures or treatment often necessitates crowding fish in small volumes of water so as to carry out the desired operations (Papadopoulos *et al*., 2008). In such instances water quality may deteriorate significantly. Having a supply of oxygen or air is essential.

Ways of reducing the negative effects of veterinary procedures

- Fish welfare must always be taken into account when implementing veterinary treatments. The usefulness and necessity of such treatments must thus be evaluated in combination with the welfare problems they could potentially cause. There are cases in which the fish's condition is irreversible, and implementing euthanasia is preferable to ineffective, painful treatment.
- Where feasible, it is helpful for treatment to be completed on part of the stock (e.g. one tank or one cage) before being continued on the remaining fish. This means that the impact on fish welfare can be assessed to decide whether or not to continue the procedure.
- Archives containing records of practices and their effects on fish welfare should be kept, to allow for possible retrospective assessment of operations aimed at improving them.

Operational Welfare Indicators when carrying out veterinary procedures

Environmental indicators	Biological indicators	
	Stock indicators	**Individual indicators**
Oxygen	Mortality	Injuries
Temperature	Behaviour	Health
pH	Injuries	Respiration rate
Stocking density		Blood glucose and lactate levels
		Blood cortisol level (laboratory indicator)

Environmental indicators

Oxygen saturation. Oxygen saturation may drop during the operations preceding sampling and in the course of veterinary procedures, which renders continuous oxygen monitoring necessary. Catching the fish initially necessitates crowding, when issues with oxygen levels may arise. Thereafter, in cases where fish are treated by bathing in small tanks that contain the therapeutic agent in solution (thus limiting the amount of medicine used) or immersion vaccinations, the fish remain in non-recycled water, where oxygen levels drop due to respiration. Oxygenation problems may likewise be observed when administrating injected vaccinations, as fish are gathered into groups from the rearing volume and placed in small, non-recirculating tanks. Oxygen must be monitored constantly throughout all of the aforementioned practices, with outside air or oxygen being supplied whenever needed. In that case, particular care must be taken not to create hyperoxic conditions in the water.

Temperature. Water temperature may vary when operations are carried out in small tanks, as mentioned above, particularly in instances where water and air temperatures differ significantly. Temperature should be monitored and steps such as changing the water or interrupting the procedure should be taken when changes are marked.

pH. In cases where fish are immersed in water containing a therapeutic agent or vaccination in solution, pH should be monitored closely to avoid any changes brought about by the agent's chemical properties. Furthermore, as the fish are placed in non-recirculating water, pH may drop due to an increase in CO_2 released during exhalation.

Stocking density. This refers to the stocking density to which fish are exposed during handling operations, e.g. immersion in water with therapeutic agents. High densities are frequently used when carrying out such operations, so as not to waste pharmaceuticals. It is thus important to record density and any issues that may arise concerning welfare and the use of other supplementary indicators, such as environmental parameters, behaviour and injuries.

Biological indicators

Mortality. Although veterinary procedures are carried out to improve health in fish, the severity of them combined with the fish's condition may cause mortality, particularly when the fish under treatment are already ill. Mortality must be recorded to evaluate whether or not treatment should be continued, as well as for retrospective examination of how severe practices are.

Behaviour. Behaviour can be evaluated on several levels, both during the husbandry procedure and in assessing the fish's recovery to normal condition once it is complete. During the practice, the emergence of "panic behaviour" or swimming behaviour can be monitored during the procedure, when the animals are not under anaesthesia. In addition to swimming behaviour, fish recovery can be assessed by monitoring the time required for feeding to return.

Injuries. Given that administering veterinary procedures involves the series of operations described above, injuries may occur. These can be monitored and recorded at stock level via the presence of scales and/or blood in the water, while macroscopic examination of fish can be used for greater accuracy.

Health. Assessing health after veterinary procedures is often carried out to monitor how successful or not treatments are, and/or the potential effects of vaccinations, and whether it is necessary to administer further treatment.

Respiration rate. As in the case of other stress-inducing practices, recording respiration rate, at least comparatively, can serve as an initial assessment of how much stress animals are subjected to.

Blood glucose and lactate levels. Sampling to determine blood glucose and lactate levels can be carried out to monitor stress in fish more precisely. Both parameters are expected to increase in fish subjected to stress.

Blood cortisol level. Cortisol is the most reliable indicator of acute stress, and can be used to evaluate the stress caused by operations carried out on fish. As a laboratory indicator it requires special laboratory equipment and appropriately trained staff.

6.2.6 | Nutrition

The nutrition strategy implemented by a farm is selected by the producer. It includes feed composition and quality, and feed delivery management with regard to quantity, frequency and distribution, as well as periods when feeding is reduced or suspended

Welfare challenges

- **Feed composition.** The composition of feed represents a significant challenge to welfare. Technology in this area is sufficiently advanced for feed to offer fish all the elements necessary for proper nourishment. Care should be taken by producers when selecting feed, so as to ensure the elements necessary for good nutrition are supplied in relation to the fish's developmental stage and physiological condition.
- **Feed delivery rate.** Feed delivery rate and frequency may affect fish welfare. In particular, meal distribution during the day plays an important role in feed utilisation, allowing for digestion of the previous meal, as does systematic rather than random feed delivery. Furthermore, proper time distribution of feed can reduce potential aggression phenomena between fish.
- **Feed delivery method.** Feed delivery method relates both to delivery systems, e.g. hand feeding, automatic or self-feeders, and to the spatial distribution of feed. Low feed dispersal has been seen to increase aggression and competition, mainly in European sea bass.
- **Fasting periods.** Fasting periods are one of the greatest challenges to welfare in feed management. Such periods usually precede operations carried out on fish such as transportation, veterinary procedures or harvesting.

Ways of reducing the negative effects of nutrition

- Recording appetite and feeding behaviour can assist in better managing nutrition.
- Competition can be averted or reduced by properly distributing the feed supplied over time and space, and feeding according to a fixed plan.

Operational Welfare Indicators during nutrition

Environmental indicators	Biological indicators	
	Stock indicators	**Individual indicators**
Oxygen	Appetite	Health
Temperature	Growth	Blood glucose level
Stocking density	Behaviour	

Environmental indicators

Oxygen saturation. Oxygen saturation can heavily influence appetite, and must thus be recorded, and feed supply appropriately adjusted where necessary.

Temperature. Temperature also directly influences feeding, and is a factor that should be taken into serious consideration in feeding tables that determine the feed supplied to a fish farm unit.

Stocking density. Both stocking density and the number of individuals being reared in a single cage or tank determine the amount of feed supplied. Consequently, the more accurate data on these parameters are, the more correct feed supply will be.

Biological indicators

Appetite. One highly significant indicator in assessing feed management is appetite. Non consumption of the feed offered on the basis of feeding tables should serve as a warning sign to monitor other welfare indicators.

Growth. The aim of nourishing fish is to increase their body weight. Good living conditions with proper nutrition in terms of feed quality and delivery management should lead to increased body weight in a healthy fish stock. Body weight growth can thus be used as an indicator of proper fish nutrition.

Behaviour. Fish behaviour during feeding can serve as an indicator of proper food delivery method. Both undernourishment and unsound food dispersal can lead to competition and aggression emerging during feeding. There should thus be a system (such as presence/absence) for evaluating such behaviours, so as to assess the procedure and improve it in retrospect whenever necessary.

Health. Fish health and vigour are both affected by feeding. A series of conditions influenced by nutrition can pose a threat to health. Firstly, in addition to weight loss, undernourishment or lengthy periods of feed deprivation may cause immune changes to an organism (Caruso *et al*., 2011), negatively affecting its health. Furthermore, a series of disorders can arise in fish due to deficiency of essential nutrients in feed, such as essential fatty acids (Montero *et al*., 2004; Skalli *et al*., 2006), amino acids, vitamins and inorganic elements including phosphorus (Kousoulaki *et al*., 2015). Lastly, nutritional diseases such as fat accumulation in the liver may arise from food composition.

Blood glucose level. Both European sea bass and gilthead sea bream show demonstrably higher levels of blood glucose postprandially, recovering to initial levels 12-24 hours later (Kousoulaki *et al*., 2015). Thus, in rare instances where detailed analysis of feed programming is required, it can serve as an indicator of meal digestion.

6.2.7| Tank and cage maintenance tasks

Simple maintenance tasks such as cleaning or painting tanks have been seen to be capable of causing stress in fish, leading to reduced feeding (Rubio *et al*., 2010). Tasks are also carried out on floating sea pens to maintain farm infrastructure and cages, e.g. nets. When carried out on cages containing fish, tasks of this type may constitute stress factors, affecting fish wellbeing.

Welfare challenges

- **Stress.** Maintenance tasks may cause stress to fish, negatively impacting their welfare.

Ways of reducing the negative effects of maintenance tasks

- Whenever possible, maintenance tasks should be carried out when fish are not being reared in the facilities concerned.

Operational welfare indicators during maintenance tasks

Environmental indicators	Biological indicators	
	Stock indicators	**Individual indicators**
Oxygen	Appetite	Injuries
	Behaviour	Health
		Respiration Rate

Environmental indicators

Oxygen saturation. As maintenance tasks may cause fish stress, thus leading to an increase in activity and respiration rate, oxygen saturation should be monitored and air or oxygen supplied as required.

Biological indicators

Appetite. Reduction or loss of appetite is one of the immediate, easily observable effects of stress. The intensity and duration of effect on feeding should thus be recorded after every maintenance task.

Behaviour. Unnatural behaviours such as "panic swimming" should be recorded whenever performing maintenance tasks.

Injuries. The unnatural swimming behaviours described above may lead to injuries due to fish striking tank walls, pen nets or each other. Observation of scales or blood in the water and macroscopic examination of the fish's condition can be used as indicators.

Health. Injuries render a fish more vulnerable to infections. As a result, any record of injuries should be followed up by monitoring the population for the potential emergence of infections.

Respiration rate. Given that respiration rate increases in fish under stress, it can be used as an indirect indicator of stress when monitoring how severe practices are.

6.2.8| Harvesting and killing

Harvesting and killing are the final stage in the production cycle. Any pain and anxiety inflicted by the killing method and the catching procedure that precedes it should be kept to a minimum throughout. Immersion in ice or ice water to provoke thermal shock and asphyxia is the killing method normally used in European sea bass and gilthead sea bream farming. Although the practice is not considered humane (van de Vis *et al.*, 2003) and does not comply with the standards laid down by the World Organisation for Animal Health (EC Final Report 2017), the European Commission [COM(2018)788 final] acknowledges that at this stage "*the Commission considers that the evidence suggests that it is not appropriate to propose specific requirements on the protection of fish at the time of killing, taking into account that the objectives of the Regulation may equally be achieved by voluntary measures, as evidenced by the improvements introduced by industry in recent years... However, it has also to be recognized that there is a need for further research aimed to tailor dedicated systems for those fish species where the development of more effective techniques is necessary.*" In recent years, the use of electrostunning has been trialled in Greece with a view to anaesthetising fish rapidly and directly after they are pumped from cages [i.e. without recourse to catching tanks and exposure to air], prior to killing via immersion in ice baths (Papaharisis *et al.*, 2019). This technology, which is used for Atlantic salmon and on a small scale for rainbow trout and carp (EC, 2018), is regarded as being in line with animal welfare demands (van de Vis *et al.*, 2003) and EU regulations (OIE, 2015), as using it guarantees immediate stunning prior to killing. At present it is being trialled to assess its suitability for future use as an alternative way of harvesting and killing. The disadvantages of this method include the fact that electrostunning must be followed up by an effective killing method, and that current levels have to be adapted and/or optimised depending on fish species and size. Lastly, unlike the commonly used killing method by immersion in ice water, electrostunning is potentially dangerous and requires staff to take strict safety measures.

Apart from killing method, the practices that precede it such as crowding, netting, exposure to air etc. have a highly significant impact on the stress inflicted and the quality of flesh. Consequently, these procedures should also be taken into account when assessing the overall effects of harvesting.

Welfare challenges

- **Pre-killing operations:** As crowding generally precedes harvesting, the challenges referred to in the appropriate section above should be taken into account. Furthermore, welfare may also be impacted by the way in which fish are transferred from cages to the killing area.
- **Killing in ice water:** Killing by immersion in a mixture of water and ice is a time-consuming procedure (Zampacavallo *et al.*, 2003), lasting up to 34 minutes until unconsciousness (Acerete *et al.*, 2009). Furthermore, when fish are in ice water, they make panic movements and attempts to escape (van de Vis *et al.*, 2003), thus reflecting reduced welfare conditions as well as the likelihood of developing injuries that will detract from end product appearance.
- **Electrostunning:** Use of an appropriate electric current depending on fish species and size. Immediate immersion in ice water to cause death before consciousness is regained.

Ways of reducing the negative effects of harvesting

- The duration and intensity of crowding prior to harvesting should be minimized, as should exposure to air during transportation.
- New methods for loading and unloading fish such as pumping should be implemented.
- New killing methods should be developed, such as electrostunning prior to killing in ice water.

Environmental Welfare Indicators during harvesting and killing

Environmental indicators	Biological indicators	
	Stock indicators	**Individual indicators**
Oxygen	Mortality	Injuries
Temperature	Behaviour	Time to rigor mortis
	Proper fish transfer	Blood and muscle pH levels
	Injuries	

Environmental indicators

Oxygen saturation. As oxygen saturation may decrease dramatically in crowding conditions, it is vital to monitor oxygen levels throughout the procedure. As a general recommendation, the EFSA report (2008) mentions that oxygen saturation in rearing conditions should be no lower than 40%.

Temperature. Temperature must be taken into account, as it can affect killing time in ice water. The temperature fish are exposed to should also be monitored, especially in the summer months, when surface water temperature may be particularly high.

Biological indicators

Mortality. In extreme instances the procedures preceding killing may cause mortality. Any such mortalities must thus be recorded, so as to pinpoint potential procedural problems in retrospect.

Behaviour. Prior to being transferred to killing tanks, the fish must exhibit calm behaviour, without making sudden movements or lashing their tails. The emergence of any such behaviours must therefore be recorded and evaluated. Thereafter, immersion in ice water is a procedure anticipated to provoke "panic behaviour" in fish, featuring intense, sudden movements. Lastly, during electrostunning the device should be monitored to ensure that all fish exiting it are unconscious.

Proper fish transfer. Transferring fish into the ice water tank involves exposing them to the air. This procedure should be minimised as far as possible, as it has a highly negative effect both on fish welfare and on end product appearance. By contrast, getting fish into an electrostunning device is usually achieved by pumping them there one by one together with water from the rearing cage. The potential for injuries to arise should be monitored during the procedure.

Injuries. Poor practices both before and during killing may cause injuries to fish. In particular, these can be incurred during crowding, transportation, exposure to air and incorrect pumping. They are normally manifested on the skin, e.g. as scale loss, and the fins, though there may also be injures to the eyes, the snout and the opercular bone.

Time to rigor mortis. Stress before killing and thus poor living conditions may lead to the faster emergence of rigor mortis. Apart from being an indicator of welfare prior to killing, this is of practical importance, as rigor mortis may create problems during product processing, such as filleting.

Blood and muscle pH levels. Blood and muscle pH can likewise be indicative of a stress-inducing event prior to death, e.g. during crowding or transportation. In particular, being under stress activates the anaerobic energy production system, which results in lactate accumulating in muscle and being released into the blood. This leads to a drop in pH, which may affect how soon rigor mortis appears and how long it lasts (Trocino *et al*., 2005; Bagni *et al*., 2007; Matos *et al*., 2010).

6.2.9| Summary

- The challenges posed to welfare may vary depending on the stage in the production cycle.
- In a similar manner, different husbandry practices have different effects on fish welfare and vigour.
- This chapter presented the challenges posed to welfare during the main stages in the production cycle and the main husbandry practices implemented, together with a description of how to use (chiefly) operational welfare indicators to monitor their effects on good living conditions.

7 Bibliography

Acerete, L., Reig, L., Alvarez, D., Flos, R., and Tort, L. 2009. Comparison of two stunning/slaughtering methods on stress response and quality indicators of European sea bass (*Dicentrarchus labrax*). Aquaculture, 287: 139–144.

Allegrucci, G., Fortunato, C., and Sbordoni, V. 1997. Genetic structure and allozyme variation of sea bass (*Dicentrarchus labrax* and *D. punctatus*) in the Mediterranean Sea. Marine Biology, 128: 347–358.

Amar, E. C., and Lavilla-Pitogo, C. R. 2004. Nutritional diseases. In Diseases of cultured groupers. Eds. by K. Nagasawa & E. R. Cruz-Lacierda. pp. 59-66. Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center.

Andrew, J. E., Holm, J., Kadri, S., and Huntingford, F. A. 2004. The effect of competition on the feeding efficiency and feed handling behaviour in gilthead sea bream (*Sparus aurata* L.) held in tanks. Aquaculture, 232: 317–331.

Anthouard, M. 1987. A Study of Social Transmission in Juvenile *Dicentrarchus labrax* (Pisces, Serranidae), in an Operant Conditioning Situation. Behaviour, 103: 266–275.

Aranda, A., Sanchez-Vázquez, F. J., and Madrid, J. A. 2001. Effect of short-term fasting on macronutrient self-selection in sea bass. Physiology & Behavior, 73: 105–109.

Araújo-Luna, R., Ribeiro, L., Bergheim, A., and Pousão-Ferreira, P. 2018. The impact of different rearing condition on gilthead seabream welfare: Dissolved oxygen levels and stocking densities. Aquaculture Research, 49: 3845–3855.

Araújo, J. E., Madeira, D., Vitorino, R., Repolho, T., Rosa, R., and Diniz, M. 2018. Negative synergistic impacts of ocean warming and acidification on the survival and proteome of the commercial sea bream, *Sparus aurata*. Journal of Sea Research, 139: 50–61.

Arechavala-Lopez, P., Diaz-Gil, C., Saraiva, J. L., Moranta, D., Castanheira, M. F., Nuñez-Velázquez, S., Ledesma-Corvi, S., *et al*. 2019. Effects of structural environmental enrichment on welfare of juvenile seabream (*Sparus aurata*). Aquaculture Reports, 15: 100224.

Ashley, P. J., Sneddon, L. U., and McCrohan, C. R. 2007. Nociception in fish: stimulus–response properties of receptors on the head of trout *Oncorhynchus mykiss*. Brain Research, 1166: 47–54.

Attia, J., Millot, S., Di-Poï, C., Bégout, M. L., Noble, C., Sanchez-Vazquez, F. J., Terova, G., *et al*. 2012. Demand feeding and welfare in farmed fish. Fish Physiology and Biochemistry, 38: 107–118.

Azeredo, R., Machado, M., Afonso, A., Fierro-Castro, C., Reyes-López, F. E., Tort, L., Gesto, M., *et al*. 2017. Neuroendocrine and immune responses undertake different fates following tryptophan or methionine dietary treatment: Tales from a teleost model. Frontiers in Immunology, 8: 1–14.

Bagni, M., Civitareale, C., Priori, A., Ballerini, A., Finoia, M., Brambilla, G., and Marino, G. 2007. Pre-slaughter crowding stress and killing procedures affecting quality and welfare in sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*). Aquaculture, 263: 52–60.

Balasch, J. C., and Tort, L. 2019. Netting the Stress Responses in Fish. Frontiers in Endocrinology, 10: 62.

Barahona-Fernandes, M. H. 1979. Some effects of light intensity and photoperiod on the sea bass larvae (*Dicentrarchus labrax* (L.)) reared at the Centre Oceanologique de Bretagne. Aquaculture, 17: 311–321.

Bardon, A., Vandeputte, M., Dupont-Nivet, M., Chavanne, H., Haffray, P., Vergnet, A., and Chatain, B. 2009. What is the heritable component of spinal deformities in the European sea bass (*Dicentrarchus labrax*)? Aquaculture, 294: 194–201.

Barton, B. A. 2002. Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology, 42: 517–525.

Barton, B. A., Ribas, L., Acerete, L., and Tort, L. 2005. Effects of chronic confinement on physiological responses of juvenile gilthead sea bream, *Sparus aurata* L., to acute handling. Aquaculture Research, 36: 172–179.

Basallote, M. D., Rodríguez-Romero, A., Blasco, J., DelValls, A., and Riba, I. 2012. Lethal effects on different marine organisms, associated with sediment–seawater acidification deriving from CO2 leakage. Environmental Science and Pollution Research, 19: 2550–2560.

Basaran, F., Ozbilgin, H., and Ozbilgin, Y. D. 2007. Effect oflordosis on the swimming performance of juvenile sea bass (*Dicentrarchus labrax* L.). Aquaculture Research, 38: 870–876.

Bavčević, L., Klanjšček, T., Karamarko, V., Aničić, I., and Legović, T. 2010. Compensatory growth in gilthead sea bream (*Sparus aurata*) compensates weight, but not length. Aquaculture, 301: 57–63.

Benhaïm, D., Péan, S., Brisset, B., Leguay, D., Bégout, M.-L., and Chatain, B. 2011. Effect of size grading on sea bass *(Dicentrarchus labrax*) juvenile self-feeding behaviour, social structure and culture performance. Aquatic Living Resources, 24: 391–402.

Bertotto, D., Poltronieri, C., Negrato, E., Richard, J., Pascoli, F., Simontacchi, C., and Radaelli, G. 2011. Whole body cortisol and expression of HSP70, IGF-I and MSTN in early development of sea bass subjected to heat shock. General and Comparative Endocrinology, 174: 44–50.

Bjerkås, E., Wall, A. E., and Prapas, A. 2000. Screening of farmed sea bass (*Dicentrarchus labrax* L) and sea bream (*Sparus aurata* L) for cataract. Bulletin of the European Association of Fish Pathologists, 20: 180–185.

Braithwaite, V. 2010. Do fish feel pain? Oxford University Press, New York. 256 pp.

Branson, E. J., and Turnbull, T. 2008. Welfare and deformities infFish. Fish Welfare: 202–216.

Broom, D. M. 1986. Indicators of poor welfare. British Veterinary Journal, 142: 524–526.

Brown, C. 2015. Fish intelligence, sentience and ethics. Animal Cognition, 18: 1–17.

Bukhari, F. A. 2005. Trials of rabbitfish, *Siganus rivulatus*, production in floating cages in the red sea. Emirates Journal of Food and Agriculture, 17: 23–29.

Caballero, M. J., Izquierdo, M. S., Kjørsvik, E., Fernández, A. J., and Rosenlund, G. 2004. Histological alterations in the liver of sea bream, *Sparus aurata* L., caused by short- or long-term feeding with vegetable oils. Recovery of normal morphology after feeding fish oil as the sole lipid source. Journal of Fish Diseases, 27: 531–541.

Cadiz, L., Zambonino-Infante, J.-L., Quazuguel, P., Madec, L., Le Delliou, H., and Mazurais, D. 2018a.

Metabolic response to hypoxia in European sea bass (*Dicentrarchus labrax*) displays developmental plasticity. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 215: 1–9. Pergamon.

Cadiz, L., Ernande, B., Quazuguel, P., Servili, A., Zambonino-Infante, J.-L., and Mazurais, D. 2018b. Moderate hypoxia but not warming conditions at larval stage induces adverse carry-over effects on hypoxia tolerance of European sea bass (*Dicentrarchus labrax*) juveniles. Marine Environmental Research, 138: 28–35. Elsevier.

Cahu, C., Infante, J. Z., and Takeuchi, T. 2003a. Nutritional components affecting skeletal development in fish larvae. Aquaculture, 227: 245–258.

Cahu, C. L., Infante, J. L. Z., and Barbosa, V. 2003b. Effect of dietary phospholipid level and phospholipid: neutral lipid value on the development of sea bass (*Dicentrarchus labrax*) larvae fed a compound diet. British Journal of Nutrition, 90: 21–28.

Can, E. 2013. Effects of intensive and semi-intensive rearing on growth, survival, and V-shaped (Lordotic) skeletal deformities in juvenile gilthead sea bream (*Sparus aurata*). Israeli Journal of Aquaculture - Bamidgeh, 65.

Carbonara, P., Dioguardi, M., Cammarata, M., Zupa, W., Vazzana, M., Spedicato, M. T., and Lembo, G. 2019a. Basic knowledge of social hierarchies and physiological profile of reared sea bass *Dicentrarchus labrax* (L.). PLOS ONE, 14: e0208688.

Carbonara, P., Alfonso, S., Zupa, W., Manfrin, A., Fiocchi, E., Pretto, T., Spedicato, M. T., *et al.* 2019b. Behavioral and physiological responses to stocking density in sea bream (*Sparus aurata*): Do coping styles matter? Physiology & Behavior, 212: 112698.

Carrillo, M., Bromage, N., Zanuy, S., Serrano, R., and Prat, F. 1989. The effect of modifications in photoperiod on spawning time, ovarian development and egg quality in the sea bass (*Dicentrarchus labrax* L.). Aquaculture, 81: 351–365.

Caruso, G., Genovese, L., Maricchiolo, G., and Modica, A. 2005. Haematological, biochemical and immunological parameters as stress indicators in *Dicentrarchus labrax* and *Sparus aurata* farmed in off-shore cages. Aquaculture International, 13: 67–73.

Caruso, G., Denaro, M. G., Caruso, R., Mancari, F., Genovese, L., and Maricchiolo, G. 2011. Response to short term starvation of growth, haematological, biochemical and non-specific immune parameters in European sea bass (*Dicentrarchus labrax*) and blackspot sea bream (*Pagellus bogaraveo*). Marine Environmental Research, 72: 46–52.

Castanheira, M. F., Herrera, M., Costas, B., Conceição, L. E. C., and Martins, C. I. M. 2013. Linking cortisol responsiveness and aggressive behaviour in gilthead seabream *Sparus aurata*: Indication of divergent coping styles. Applied Animal Behaviour Science, 143: 75–81.

Cataudella, S., Allegrucci, G., Bronzi, P., Castaldi, E., Cioni, C., Crosetti, D., De-Merich, D., *et al.* 1991. Multidisciplinary approach to the optimisation of seabass (*Dicentrarchus labrax*) rearing in freshwater. Aquaculture and the Environment - EAS Special Publication, 14: 656–661.

Cecchini, S., and Saroglia, M. 2002. Antibody response in sea bass (*Dicentrarchus labrax* L.) in relation to water temperature and oxygenation. Aquaculture Research, 33: 607–613.

Cecchini, S., and Caputo, A. R. 2003. Acid-base balance in sea bass (*Dicentrarchus labrax* L.) in relation to water oxygen concentration. Aquaculture Research, 34: 1069–1073.

Cerdá-Reverter, J. M., Zanuy, S., Carrillo, M., and Madrid, J. A. 1998. Time-course studies on plasma glucose, insulin, and cortisol in sea bass (*Dicentrarchus labrax*) held under different photoperiodic regimes. Physiology and Behavior, 64: 245–250.

Cerdá, J., Carrillo, M., Zanuy, S., Ramos, J., and de la Higuera, M. 1994. Influence of nutritional composition of diet on sea bass, *Dicentrarchus labrax* L., reproductive performance and egg and larval quality. Aquaculture, 128: 345–361.

Chatain, B. 1994. Abnormal swimbladder development and lordosis in sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus auratus*). Aquaculture, 119: 371–379.

Chatzifotis, S., Papadaki, M., Despoti, S., Roufidou, C., and Antonopoulou, E. 2011. Effect of starvation and re-feeding on reproductive indices, body weight, plasma metabolites and oxidative enzymes of sea bass (*Dicentrarchus labrax*). Aquaculture, 316: 53–59.

Claireaux, G., and Lagardère, J. P. 1999. Influence of temperature, oxygen and salinity on the metabolism of the European sea bass. Journal of Sea Research, 42: 157–168.

Claireaux, G., and Chabot, D. 2016. Responses by fishes to environmental hypoxia: Integration through Fry's concept of aerobic metabolic scope. Journal of Fish Biology, 88: 232–251.

Cominassi, L., Moyano, M., Claireaux, G., Howald, S., Mark, F. C., Zambonino-Infante, J.-L., Le Bayon, N., *et al.* 2019. Combined effects of ocean acidification and temperature on larval and juvenile growth, development and swimming performance of European sea bass (*Dicentrarchus labrax*). PLOS ONE, 14: e0221283.

Conides, A. J., and Glamuzina, B. 2001. Study on the effects of rearing density, temperature and salinity on hatching performance of the European sea bass, *Dicentrarchus labrax* (Linnaeus, 1758). Aquaculture International, 9: 217–224.

Cooke, M. 2016. BBFAW Animal Welfare in Farmed Fish: 1–16.

Crespel, A., Zambonino-Infante, J.-L., Mazurais, D., Koumoundouros, G., Fragkoulis, S., Quazuguel, P., Huelvan, C., *et al.* 2017. The development of contemporary European sea bass larvae (*Dicentrarchus labrax*) is not affected by projected ocean acidification scenarios. Marine Biology, 164: 155.

Cuesta, A., Laiz-Carrión, R., Martín Del Río, M. P., Meseguer, J., Miguel Mancera, J., and Ángeles Esteban, M. 2005. Salinity influences the humoral immune parameters of gilthead seabream (*Sparus aurata* L.). Fish and Shellfish Immunology, 18: 255–261.

Cuvier-Péres, A., Jourdan, S., Fontaine, P., and Kestemont, P. 2001. Effects of light intensity on animal husbandry and digestive enzyme activities in sea bass *Dicentrarchus labrax* post-larvae. Aquaculture, 202: 317–328.

Dalla Via, J., Villani, P., Gasteiger, E., and Niederstätter, H. 1998. Oxygen consumption in sea bass fingerling *Dicentrarchus labrax* exposed to acute salinity and temperature changes: Metabolic basis for maximum stocking density estimations. Aquaculture, 169: 303–313.

Di Marco, P., Priori, A., Finoia, M. G., Massari, A., Mandich, A., and Marino, G. 2008. Physiological responses of European sea bass *Dicentrarchus labrax* to different stocking densities and acute stress challenge. Aquaculture, 275: 319–328.

Divanach, P., Papandroulakis, N., Anastasiadis, P., Koumoundouros, G., and Kentouri, M. 1997. Effect of water currents on the development of skeletal deformities in sea bass (*Dicentrarchus labrax* L.) with functional swimbladder during postlarval and nursery phase. Aquaculture, 156: 145–155.

Dülger, N., Kumlu, M., Türkmen, S., Ölçülü, A., Tufan Eroldoğan, O., Asuman Yılmaz, H., and Öçal, N. 2012. Thermal tolerance of european sea bass (*Dicentrarchus labrax*) juveniles acclimated to three temperature levels. Journal of Thermal Biology, 37: 79–82.

Duteil, M., Pope, E. C., Pérez-Escudero, A., de Polavieja, G. G., Fürtbauer, I., Brown, M. R., and King, A. J. 2016. European sea bass show behavioural resilience to near-future ocean acidification. Royal Society Open Science, 3: 160656.

EC. 2009. Expert working group on severity classification of scientific procedures performed on animals. Expert Working Group of the European Union.

EC. 2018. REPORT FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT AND THE COUNCIL on the possibility of introducing certain requirements regarding the protection of fish at the time of killing. European Commission: 1–14.

EFSA. 2008. Scientific Opinion of the Panel on Animal Health and Welfare on a request from the European Commission on animal welfare aspects of husbandry systems for farmed European seabass and Gilthead seabream. The EFSA journal: 1–21.

Eleftheriou, M. 1998. Aqualex: a glossary of aquaculture terms. 410 pp.

Ellis, T., Yildiz, H. Y., López-Olmeda, J., Spedicato, M. T., Tort, L., Øverli, Ø., and Martins, C. I. M. 2012. Cortisol and finfish welfare. Fish Physiology and Biochemistry, 38: 163–188.

Enes, P., Panserat, S., Kaushik, S., and Oliva-Teles, A. 2011. Dietary carbohydrate utilization by European Sea Bass (*Dicentrarchus labrax* L.) and gilthead sea bream (*Sparus aurata* L.) Juveniles. Reviews in Fisheries Science, 19: 201–215.

Eroldoğan, O. T., and Kumlu, M. 2002. Growth performance, body traits and fillet composition of the European sea bass (*Dicentrarchus labrax*) reared in various salinities and fresh water. Turkish Journal of Veterinary and Animal Sciences, 26: 993–1001.

Eroldoğan, O. T., Kumlu, M., and Aktaş, M. 2004. Optimum feeding rates for European sea bass *Dicentrarchus labrax* L. reared in seawater and freshwater. Aquaculture, 231: 501–515.

Eroldoğan, O. T., Kumlu, M., Kiris, G. A., and Sezer, B. 2006. Compensatory growth response of *Sparus aurata* following different starvation and refeeding protocols. Aquaculture Nutrition, 12: 203–210.

Fanouraki, E., Mylonas, C. C., Papandroulakis, N., and Pavlidis, M. 2011. Species specificity in the magnitude and duration of the acute stress response in Mediterranean marine fish in culture. General and Comparative Endocrinology, 173: 313–322.

FAO. 2005. Cultured Aquatic Species Information Programme. *Sparus aurata*. Cultured Aquatic Species Information Programme.

Fazio, F., Ferrantelli, V., Fortino, G., Arfuso, F., Giangrosso, G., and Faggio, C. 2015. The influence of acute handling stress on some blood parameters in cultured sea bream (*Sparus aurata*, Linnaeus, 1758). Italian Journal of Food Safety, 4: 4–6.

Fazio, F., Ferrantelli, V., Piccione, G., Saoca, C., Levanti, M., and Mucciardi, M. 2018. Biochemical and hematological parameters in European sea bass (*Dicentrarchus labrax* Linnaeus, 1758) and gilthead sea bream (*Sparus aurata* Linnaeus, 1758) in relation to temperature. Veterinarski Arhiv, 88: 397–411.

Felip, A., Zanuy, S., Muriach, B., Cerdá-Reverter, J. M., and Carrillo, M. 2008. Reduction of sexual maturation in male *Dicentrarchus labrax* by continuous light both before and during gametogenesis. Aquaculture, 275: 347–355.

Ferreira Pinto, J., Nunes, M. L., and Cardoso, C. 2007. Feeding interruption and quality of cultured gilthead sea bream. Food Chemistry, 100: 1504–1510.

Fraser, D. 2008. Understanding animal welfare. Acta Veterinaria Scandinavica, 50: S1.

García-Celdrán, M., Ramis, G., Manchado, M., Estévez, A., Afonso, J. M., María-Dolores, E., Peñalver, J., *et al*. 2015. Estimates of heritabilities and genetic correlations of growth and external skeletal deformities at different ages in a reared gilthead sea bream (*Sparus aurata* L.) population sourced from three broodstocks along the Spanish coasts. Aquaculture, 445: 33–41.

Georgakopoulou, E., Angelopoulou, A., Kaspiris, P., Divanach, P., and Koumoundouros, G. 2007. Temperature effects on cranial deformities in European sea bass, *Dicentrarchus labrax* (L.). Journal of Applied Ichthyology, 23: 99–103.

Georgakopoulou, E., Katharios, P., Divanach, P., and Koumoundouros, G. 2010. Effect of temperature on the development of skeletal deformities in Gilthead seabream (*Sparus aurata* Linnaeus, 1758). Aquaculture, 308: 13–19.

Ginés, R., Afonso, J. M., Argüello, A., Zamorano, M. J., and López, J. L. 2003. Growth in adult gilthead sea bream (*Sparus aurata* L) as a result of interference in sexual maturation by different photoperiod regimes. Aquaculture Research, 34: 73–83.

Ginés, R., Afonso, J. M., Argüello, A., Zamorano, M. J., and López, J. L. 2004. The effects of long-day photoperiod on growth, body composition and skin colour in immature gilthead sea bream (*Sparus aurata* L.). Aquaculture Research, 35: 1207–1212.

Goda, A. M. A. S., Srour, T. M., Mansour, A. T., Baromh, M. Z., Sallam, G. R., and Baromh, A. Z. 2019. Assessment of stresful ambient water salinity on growth, feed utilization and hematological indices of european sea bass, *Dicentrarchus labrax*, juveniles. AACL Bioflux, 12: 553–563.

Goldan, O., Popper, D., and Karplus, I. 2003. Food competition in small groups of juvenile gilthead sea bream (*Sparus aurata*). Israeli Journal of Aquaculture - Bamidgeh, 55: 94–106.

Gómez-Laplaza, L. M., and Gerlai, R. 2011. Can angelfish (*Pterophyllum scalare*) count? Discrimination between different shoal sizes follows Weber's law. Animal Cognition, 14: 1–9.

Gómez-Milán, E., de Haro, C., and Sánchez-Muros, M. J. 2011. Annual variations of the plasmatic levels of glucose and amino acid and daily changes under different natural conditions of temperature and photoperiod in Gilthead Sea bream (*Sparus aurata*, L.). Fish Physiology and Biochemistry, 37: 583–592.

Guardiola, F. A., Cuesta, A., and Esteban, M. Á. 2016. Using skin mucus to evaluate stress in gilthead seabream (*Sparus aurata* L.). Fish & Shellfish Immunology, 59: 323–330.

Güçlüsoy, H., and Savas, Y. 2003. Interaction between monk seals *Monachus monachus* (Hermann, 1779) and marine fish farms in the Turkish Aegean and management of the problem. Aquaculture Research, 34: 777–783.

Hastein, T. 2004. Animal welfare issues relating to aquaculture. In Proceedings of the Global Conference on Animal Welfare: an OIE initiative., pp. 219–231.

Hatziathanasiou, A., Paspatis, M., Houbart, M., Kestemont, P., Stefanakis, S., and Kentouri, M. 2002. Survival, growth and feeding in early life stages of European sea bass (*Dicentrarchus labrax*) intensively cultured under different stocking densities. Aquaculture, 205: 89–102.

Hawkins, P., Dennison, N., Goodman, G., Hetherington, S., Llywelyn-Jones, S., Ryder, K., and Smith, A. J. 2011. Guidance on the severity classification of scientific procedures involving fish: Report of a Working Group appointed by the Norwegian Consensus-Platform for the Replacement, Reduction and Refinement of animal experiments (Norecopa). Laboratory Animals, 45: 219–224.

Humane Society International. 2012. The Welfare of Animals in the Aquaculture Industry: 24.

Huntingford, F. A., Adams, C., Braithwaite, V. A., Kadri, S., Pottinger, T. G., Sandoe, P., and Turnbull, J. F. 2006. Current issues in fish welfare. Journal of Fish Biology, 68: 332–372.

Jerez-Cepa, I., Fernández-Castro, M., Del Santo O'Neill, T. J., Martos-Sitcha, J. A., Martínez-Rodríguez, G., Mancera, J. M., and Ruiz-Jarabo, I. 2019. Transport and recovery of gilthead seabream (*Sparus aurata* L.) sedated with clove oil and MS-222: Effects on stress axis regulation and intermediary metabolism. Frontiers in Physiology, 10.

Johnson, D. V., and Katavic, I. 1984. Mortality, growth and swim bladder stress syndrome of sea bass. Aquaculture, 38: 67–78.

Karahan, B., Chatain, B., Chavanne, H., Vergnet, A., Bardon, A., Haffray, P., Dupont-Nivet, M., *et al*. 2013. Heritabilities and correlations of deformities and growth-related traits in the European sea bass (*Dicentrarchus labrax*, L) in four different sites. Aquaculture Research, 44: 289–299.

Karakatsouli, N., Papoutsoglou, S. E., Pizzonia, G., Tsatsos, G., Tsopelakos, A., Chadio, S., Kalogiannis, D., *et al*. 2007. Effects of light spectrum on growth and physiological status of gilthead seabream *Sparus aurata* and rainbow trout *Oncorhynchus mykiss* reared under recirculating system conditions. Aquacultural Engineering, 36: 302–309.

Key, B. 2016. Why fish do not feel pain. Animal Sentience, 3: 1–33.

Kissil, G. W., Lupatsch, I., Elizur, A., and Zohar, Y. 2001. Long photoperiod delayed spawning and increased somatic growth in gilthead seabream (*Sparus aurata*). Aquaculture, 200: 363–379.

Kleinhappel, T. K., Pike, T. W., and Burman, O. H. P. 2019. Stress-induced changes in group behaviour. Scientific Reports, 9: 17200.

Koumoundouros, G. 2002. Effect of temperature on swimming performance of sea bass juveniles. Journal of Fish Biology, 60: 923–932.

Kousoulaki, K., Sether, B. S., Albrektsen, S., and Noble, C. 2015. Review on European sea bass (*Dicentrarchus labrax*, Linnaeus, 1758) nutrition and feed management: A practical guide for optimizing feed formulation and farming protocols. Aquaculture Nutrition, 21: 129–151.

Laiz-Carrión, R., Sangiao-Alvarellos, S., Guzmán, J. M., Martín Del Río, M. P., Soengas, J. L., and Mancera, J. M. 2005. Growth performance of gilthead sea bream *Sparus aurata* in different osmotic conditions: Implications for osmoregulation and energy metabolism. Aquaculture, 250: 849–861.

Leal, E., Fernández-Durán, B., Guillot, R., Ríos, D., and Cerdá-Reverter, J. M. 2011. Stress-induced effects on feeding behavior and growth performance of the sea bass (*Dicentrarchus labrax*): A self-feeding approach. Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology, 181: 1035–1044.

Lee-Montero, I., Navarro, A., Negrín-Báez, D., Zamorano, M. J., Berbel, C., Sánchez, J. A., García-Celdran, M., *et al*. 2015. Genetic parameters and genotype-environment interactions for skeleton deformities and growth traits at different ages on gilthead seabream (*Sparus aurata* L.) in four Spanish regions. Animal Genetics, 46: 164–174.

Lemarié, G., Hosfeld, C. D., Breuil, G., and Fivelstad, S. 2011. Effects of hyperoxic water conditions under different total gas pressures in European sea bass (*Dicentrarchus labrax*). Aquaculture, 318: 191–198.

Lika, K., Pavlidis, M., Mitrizakis, N., Samaras, A., and Papandroulakis, N. 2015. Do experimental units of different scale affect the biological performance of European sea bass *Dicentrarchus labrax* larvae? Journal of Fish Biology, 86: 1271–1285.

López-Olmeda, J. F., Noble, C., and Sánchez-Vázquez, F. J. 2012. Does feeding time affect fish welfare? Fish Physiology and Biochemistry, 38: 143–152.

Lupatsch, I., Santos, G. A., Schrama, J. W., and Verreth, J. A. J. 2010. Effect of stocking density and feeding level on energy expenditure and stress responsiveness in European sea bass *Dicentrarchus labrax*. Aquaculture, 298: 245–250.

Lupi, P., Vigiani, V., Mecatti, M., and Bozzi, R. 2005. First haematic results for the sea bass (*Dicentrarchus labrax*) metabolic profile assessment. Italian Journal of Animal Science, 4: 167–176.

Mabrouk, H. A., and Nour, A. M. 2011. Effect of reducing water salinity on survival, growth performance, chemical composition and nutrients gain of gilthead sea bream *Sparus aurata* larvae. Journal of King Abdulaziz University, Marine Science, 22: 15–29.

Madeira, D., Costa, P. M., Vinagre, C., and Diniz, M. S. 2016. When warming hits harder: survival, cellular stress and thermal limits of *Sparus aurata* larvae under global change. Marine Biology, 163: 1–14.

Magnoni, L. J., Martos-Sitcha, J. A., Queiroz, A., Calduch-Giner, J. A., Gonçalves, J. F. M., Rocha, C. M. R., Abreu, H. T., *et al*. 2017. Dietary supplementation of heat-treated Gracilaria and Ulva seaweeds enhanced acute hypoxia tolerance in gilthead sea bream (*Sparus aurata*). Biology Open, 6: 897–908.

Makridis, P., Mente, E., Grundvig, H., Gausen, M., Koutsikopoulos, C., and Bergheim, A. 2018. Monitoring of oxygen fluctuations in seabass cages (*Dicentrarchus labrax* L.) in a commercial fish farm in Greece. Aquaculture Research, 49: 684–691.

Mañanós, E. L., Zanuy, S., and Carrillo, M. 1997. Photoperiodic manipulations of the reproductive cycle of sea bass (*Dicentrarchus labrax*) and their effects on gonadal development, and plasma 17β-estradiol and vitellogenin levels. Fish Physiology and Biochemistry, 16: 211–222.

Mancera, J. M., Vargas-Chacoff, L., García-López, A., Kleszczyńska, A., Kalamarz, H., Martínez-Rodríguez, G., and Kulczykowska, E. 2008. High density and food deprivation affect arginine vasotocin, isotocin and melatonin in gilthead sea bream (*Sparus auratus*). Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology, 149: 92–97.

Manuel, R., Gorissen, M., Zethof, J., Ebbesson, L. O. E., van de Vis, H., Flik, G., and Van Den Bos, R. 2014. Unpredictable chronic stress decreases inhibitory avoidance learning in Tuebingen long-fin zebrafish:

Stronger effects in the resting phase than in the active phase. Journal of Experimental Biology, 217: 3919–3928.

Marino, G., Cataldi, E., Pucci, P., and Cataudella, S. 1994. Acclimation trials of wild and hatchery sea bass (*Dicentrarchus labrax*) fry at different salinities. Journal of Applied Ichthyology, 10: 57–63.

Martins, C. I. M., Galhardo, L., Noble, C., Damsgård, B., Spedicato, M. T., Zupa, W., Beauchaud, M., *et al*. 2012. Behavioural indicators of welfare in farmed fish. Fish Physiology and Biochemistry, 38: 17–41.

Martos-Sitcha, J. A., Bermejo-Nogales, A., Calduch-Giner, J. A., and Pérez-Sánchez, J. 2017. Gene expression profiling of whole blood cells supports a more efficient mitochondrial respiration in hypoxia-challenged gilthead sea bream (*Sparus aurata*). Frontiers in Zoology, 14: 34.

Martos-Sitcha, J. A., Sosa, J., Ramos-Valido, D., Bravo, F. J., Carmona-Duarte, C., Gomes, H. L., Calduch-Giner, J. À., *et al*. 2019. Ultra-low power sensor devices for monitoring physical activity and respiratory frequency in farmed fish. Frontiers in Physiology, 10: 1–14.

Masroor, W., Farcy, E., Gros, R., and Lorin-Nebel, C. 2018. Effect of combined stress (salinity and temperature) in European sea bass *Dicentrarchus labrax* osmoregulatory processes. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 215: 45–54.

Matos, E., Gonçalves, A., Nunes, M. L., Dinis, M. T., and Dias, J. 2010. Effect of harvesting stress and slaughter conditions on selected flesh quality criteria of gilthead seabream (*Sparus aurata*). Aquaculture, 305: 66–72.

Maximino, C., Marques de Brito, T., Dias, C. A. G. de M., Gouveia, A., and Morato, S. 2010. Scototaxis as anxiety-like behavior in fish. Nature Protocols, 5: 209–216.

Milligan, C. L. 1996. Metabolic recovery from exhaustive exercise in rainbow trout. Comparative Biochemistry and Physiology - A Physiology, 113: 51–60.

Millot, S., and Bégout, M.-L. 2009. Individual fish rhythm directs group feeding: a case study with sea bass juveniles (*Dicentrarchus labrax*) under self-demand feeding conditions. Aquatic Living Resources, 22: 363–370.

Millot, S., Cerqueira, M., Castanheira, M.-F., Øverli, Ø., Oliveira, R. F., and Martins, C. I. M. 2014. Behavioural Stress Responses Predict Environmental Perception in European Sea Bass (*Dicentrarchus labrax*). PLoS ONE, 9: e108800.

Mohammed-Geba, K., Yúfera, M., Martínez-Rodríguez, G., and Mancera, J. M. 2016. Molecular endocrine changes of Gh/Igf1 axis in gilthead sea bream (*Sparus aurata* L.) exposed to different environmental salinities during larvae to post-larvae stages. Fish Physiology and Biochemistry, 42: 1177–1186.

Mommsen, T. P., Vijayan, M. M., and Moon, T. W. 1999. Cortisol in teleosts: Dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology and Fisheries, 9: 211–268.

Montero, D., Izquierdo, M. S., Tort, L., Robaina, L., and Vergara, J. M. 1999. High stocking density produces crowding stress altering some physiological and biochemical parameters in gilthead seabream, *Sparus aurata*, juveniles. Fish Physiology and Biochemistry, 20: 53–60.

Montero, D., Socorro, J., Tort, L., Caballero, M. J., Robaina, L. E., Vergara, J. M., and Izquierdo, M. S. 2004. Glomerulonephritis and immunosuppression associated with dietary essential fatty acid deficiency in gilthead sea bream, *Sparus aurata* L., juveniles. Journal of Fish Diseases, 27: 297–306.

Montero, D., Lalumera, G., Izquierdo, M. S., Caballero, M. J., Saroglia, M., and Tort, L. 2009. Establishment of dominance relationships in gilthead sea bream *Sparus aurata* juveniles during feeding: Effects on feeding behaviour, feed utilization and fish health. Journal of Fish Biology, 74: 790–805.

Montoya, A., López-Olmeda, J. F., Garayzar, A. B. S., and Sánchez-Vázquez, F. J. 2010. Synchronization of daily rhythms of locomotor activity and plasma glucose, cortisol and thyroid hormones to feeding in Gilthead seabream (*Sparus aurata*) under a light–dark cycle. Physiology & Behavior, 101: 101–107.

Moyano, M., Candebat, C., Ruhbaum, Y., Álvarez-Fernández, S., Claireaux, G., Zambonino-Infante, J. L., and Peck, M. A. 2017. Effects of warming rate, acclimation temperature and ontogeny on the critical thermal maximum of temperate marine fish larvae. PLoS ONE, 12: 1–23.

Mylonas, C. C., Sigelaki, I., Divanach, P., Mananõs, E., Carrillo, M., and Afonso-Polyviou, A. 2003. Multiple spawning and egg quality of individual European sea bass (*Dicentrarchus labrax*) females after repeated injections of GnRHa. Aquaculture, 221: 605–620.

Mylonas, C. C., Papandroulakis, N., Smboukis, A., Papadaki, M., and Divanach, P. 2004. Induction of spawning of cultured greater amberjack (*Seriola dumerili*) using GnRHa implants. Aquaculture, 237: 141–154.

Nash, C. E., Iwamoto, R. N., and Mahnken, C. V. W. 2000. Aquaculture risk management and marine mammal interactions in the Pacific Northwest. Aquaculture, 183: 307–323.

Negrín-Báez, D., Navarro, A., Lee-Montero, I., Soula, M., Afonso, J. M., and Zamorano, M. J. 2015a. Inheritance of skeletal deformities in gilthead seabream (*Sparus aurata*) –lack of operculum, lordosis, vertebral fusion and LSK complex. Journal of Animal Science, 93: 53–61.

Negrín-Báez, D., Navarro, A., Afonso, J. M., Ginés, R., and Zamorano, M. J. 2015b. Detection of QTL associated with three skeletal deformities in gilthead seabream (*Sparus aurata* L.): Lordosis, vertebral fusion and jaw abnormality. Aquaculture, 448: 123–127. Elsevier B.V.

Noble, C., Hernan, J., Damsgard, B., Flood, M., Midling, K., Roque, A., Saether, B.-S., *et al*. 2012. Injuries and deformities in fish : their potential impacts upon aquacultural production and welfare: 61–83.

Noble, C., Gismervik, K., Iversen, M. H., Kolarevic, J., Nilsson, J., Stien, L. H., and Turnbull, J. F. 2018. Welfare Indicators for farmed Atlantic salmon: tools for assessing fish welfare. 351 pp.

OIE, W. O. for A. H. 2015. Welfare aspects of animal stunning and killing of farmed fish for human consumption. Aquatic Animal Health Code.

Oikonomidou, E., Batzina, A., and Karakatsouli, N. 2019. Effects of food quantity and distribution on aggressive behaviour of gilthead seabream and European seabass. Applied Animal Behaviour Science, 213: 124–130.

Papadakis, V. M., Glaropoulos, A., Alvanopoulou, M., and Kentouri, M. 2016. A behavioural approach of dominance establishment in tank-held sea bream (*Sparus aurata* L.) under different feeding conditions. Aquaculture Research, 47: 4015–4023.

Papadopoulos, P., Bitchava, K., Tzironi, E., and Athanassopoulou, F. 2008. Fish Vaccination. Journal of the Hellenic Veterinary Medical Society, 59: 308–319.

Papaharisis, L., Tsironi, T., Dimitroglou, A., Taoukis, P., and Pavlidis, M. 2019. Stress assessment, quality indicators and shelf life of three aquaculture important marine fish, in relation to harvest practices, water temperature and slaughter method. Aquaculture Research, 50: 2608–2620.

Papandroulakis, N., Divanach, P., Anastasiadis, P., and Kentouri, M. 2001. The pseudo-green water technique for intensive rearing of sea bream (*Sparus aurata*) larvae. Aquaculture International, 9: 205–216.

Papandroulakis, N., Lika, K., Kristiansen, T. S., Oppedal, F., Divanach, P., and Pavlidis, M. 2014. Behaviour of European sea bass, *Dicentrarchus labrax* L., in cages - impact of early life rearing conditions and management. Aquaculture Research, 45: 1545–1558.

Pascoli, F., Lanzano, G. S., Negrato, E., Poltronieri, C., Trocino, A., Radaelli, G., and Bertotto, D. 2011. Seasonal effects on hematological and innate immune parameters in sea bass *Dicentrarchus labrax*. Fish and Shellfish Immunology, 31: 1081–1087.

Paspatis, M., Maragoudaki, D., and Kentouri, M. 2000. Self-feeding activity patterns in gilthead sea bream (*Sparus aurata*), red porgy (*Pagrus pagrus*) and their reciprocal hybrids. Aquaculture, 190: 389–401.

Pavlidis, M., Berry, M., Divanach, P., and Kentouri, M. 1997. Diel pattern of haematocrit, serum metabolites, osmotic pressure, electrolytes and thyroid hormones in sea bass and sea bream. Aquaculture International, 5: 237–247.

Pavlidis, M., Koumoundouros, G., Sterioti, A., Somarakis, S., Divanach, P., and Kentouri, M. 2000. Evidence of temperature-dependent sex determination in the european sea bass (*Dicentrarchus labrax* L.). Journal of Experimental Zoology, 287: 225–232.

Peixoto, M. J., Svendsen, J. C., Malte, H., Pereira, L. F., Carvalho, P., Pereira, R., Gonçalves, J. F. M., *et al.* 2016. Diets supplemented with seaweed affectmetabolic rate, innate immune, and antioxidant responses, but not individual growth rate in European seabass (*Dicentrarchus labrax*). Journal of Applied Phycology, 28: 2061–2071.

Peres, H., and Oliva-Teles, A. 1999a. Influence of temperature on protein utilization in juvenile European seabass (*Dicentrarchus labrax*). Aquaculture, 170: 337–348.

Peres, H., and Oliva-Teles, A. 1999b. Effect of dietary lipid level on growth performance and feed utilization by European sea bass juveniles (*Dicentrarchus labrax*). Aquaculture, 179: 325–334.

Peres, H., Gonçalves, P., and Oliva-Teles, A. 1999. Glucose tolerance in gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*). Aquaculture, 179: 415–423.

Peres, H., Santos, S., and Oliva-Teles, A. 2011. Lack of compensatory growth response in gilthead seabream (*Sparus aurata*) juveniles following starvation and subsequent refeeding. Aquaculture, 318: 384–388.

Person-Le Ruyet, J., Mahé, K., Le Bayon, N., and Le Delliou, H. 2004. Effects of temperature on growth and metabolism in a Mediterranean population of European sea bass, *Dicentrarchus labrax*. Aquaculture, 237: 269–280.

Person-Le Ruyet, J., and Le Bayon, N. 2009. Effects of temperature, stocking density and farming conditions on fin damage in European sea bass (*Dicentrarchus labrax*). Aquatic Living Resources, 22: 349–362.

Petochi, T., Di Marco, P., Priori, A., Finoia, M. G., Mercatali, I., and Marino, G. 2011. Coping strategy and stress response of European sea bass *Dicentrarchus labrax* to acute and chronic environmental hypercapnia under hyperoxic conditions. Aquaculture, 315: 312–320.

Pichavant, K., Person-Le-Ruyet, J., Le Bayon, N., Severe, A., Le Roux, A., and Boeuf, G. 2001. Comparative effects of long-term hypoxia on growth, feeding and oxygen consumption in juvenile turbot and European sea bass. Journal of Fish Biology, 59: 875–883.

Piferrer, F., Blázquez, M., Navarro, L., and González, A. 2005. Genetic, endocrine, and environmental components of sex determination and differentiation in the European sea bass (*Dicentrarchus labrax* L.). General and Comparative Endocrinology, 142: 102–110.

Pimentel, M. S., Faleiro, F., Marques, T., Bispo, R., Dionísio, G., Faria, A. M., Machado, J., *et al.* 2016. Foraging behaviour, swimming performance and malformations of early stages of commercially important fishes under ocean acidification and warming. Climatic Change, 137: 495–509.

Poulton, D., Porteus, C. S., and Simpson, S. 2017. Combined impacts of elevated CO2 and anthropogenic noise on European sea bass (*Dicentrarchus labrax*). ICES Journal of Marine Science, 74: 1230–1236.

Prestinicola, L., Boglione, C., Makridis, P., Spanò, A., Rimatori, V., Palamara, E., Scardi, M., *et al.* 2013. Environmental Conditioning of Skeletal Anomalies Typology and Frequency in Gilthead Seabream (*Sparus aurata* L., 1758) Juveniles. PLoS ONE, 8: e55736.

Rachels, J., and Rachels, S. 2010. The elements of moral philosophy. Εκδόσεις Οκτώ, Αθήνα.

Regan, T. 1983. The Case for Animal Rights. University of California Press, Berkeley.

Réveillac, E., Lacoue-Labarthe, T., Oberhänsli, F., Teyssié, J.-L., Jeffree, R., Gattuso, J.-P., and Martin, S. 2015. Ocean acidification reshapes the otolith-body allometry of growth in juvenile sea bream. Journal of Experimental Marine Biology and Ecology, 463: 87–94.

Robaina, L., Corraze, G., Aguirre, P., Blanc, D., Melcion, J. P., and Kaushik, S. 1999. Digestibility, postprandial ammonia excretion and selected plasma metabolites in European sea bass (*Dicentrarchus labrax*) fed pelleted or extruded diets with or without wheat gluten. Aquaculture, 179: 45–56.

Rodríguez, L., Begtashi, I., Zanuy, S., and Carrillo, M. 2005. Long-term exposure to continuous light inhibits precocity in European male sea bass (*Dicentrarchus labrax*, L.): Hormonal aspects. General and Comparative Endocrinology, 140: 116–125.

Rose, J. D. 2002. The Neurobehavioral Nature of Fishes and the Question of Awareness and Pain. Reviews in Fisheries Science, 10: 1–38.

Rose, J. D., Arlinghaus, R., Cooke, S. J., Diggles, B. K., Sawynok, W., Stevens, E. D., and Wynne, C. D. L. 2014. Can fish really feel pain? Fish and Fisheries, 15: 97–133.

Rotllant, J., Balm, P. H. M., Ruane, N. M., Pérez-Sánchez, J., Wendelaar-Bonga, S. E., and Tort, L. 2000a. Pituitary proopiomelanocortin-derived peptides and hypothalamus-pituitary-interrenal axis activity in gilthead sea bream (*Sparus aurata*) during prolonged crowding stress: Differential regulation of adrenocorticotropin hormone and α-melanocyte-stimulating. General and Comparative Endocrinology, 119: 152–163.

Rotllant, J., Balm, P. H. M., Ruane, N. M., Pérez-Sánchez, J., Wendelaar-Bonga, S. E., and Tort, L. 2000b. Pituitary proopiomelanocortin-derived peptides and Hypothalamus–Pituitary–Interrenal axis activity in gilthead sea bream (*Sparus aurata*) during prolonged crowding stress: Differential regulation of adrenocorticotropin hormone and α-Melanocyte-Stimulating. General and Comparative Endocrinology, 119: 152–163.

Rotllant, J., Balm, P. H. M., Pérez-Sánchez, J., Wendelaar-Bonga, S. E., and Tort, L. 2001. Pituitary and interrenal function in gilthead sea bream (*Sparus aurata* L., Teleostei) after handling and confinement stress. General and Comparative Endocrinology, 121: 333–342.

Rotllant, J., Ruane, N. M., Caballero, M. J., Montero, D., and Tort, L. 2003. Response to confinement in sea bass (*Dicentrarchus labrax*) is characterised by an increased biosynthetic capacity of interrenal tissue with no effect on ACTH sensitivity. Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology, 136: 613–620.

Rubio, V. C., Vivas, M., Sánchez-Mut, A., Sánchez-Vázquez, F. J., Covès, D., Dutto, G., and Madrid, J. A. 2004. Self-feeding of European sea bass (*Dicentrarchus labrax*, L.) under laboratory and farming conditions using a string sensor. Aquaculture, 233: 393–403.

Rubio, V. C., Sánchez, E., and Cerdá-Reverter, J. M. 2010. Compensatory feeding in the sea bass after fasting and physical stress. Aquaculture, 298: 332–337.

Russell, N. R., Fish, J. D., and Wootton, R. J. 1996. Feeding and growth of juvenile sea bass: The effect of ration and temperature on growth rate and efficiency. Journal of Fish Biology, 49: 206–220.

Saillant, E., Fostier, A., Haffray, P., Menu, B., Laureau, S., Thimonier, J., and Chatain, B. 2003. Effects of rearing density, size grading and parental factors on sex ratios of the sea bass (*Dicentrarchus labrax* L.) in intensive aquaculture. Aquaculture, 221: 183–206.

Samaras, A., Papandroulakis, N., Costari, M., and Pavlidis, M. 2016. Stress and metabolic indicators in a relatively high (European sea bass, *Dicentrarchus labrax*) and a low (meagre, *Argyrosomus regius*) cortisol responsive species, in different water temperatures. Aquaculture Research, 47: 3501–3515.

Samaras, A., Pavlidis, M., Lika, K., Theodoridi, A., and Papandroulakis, N. 2017. Scale matters: performance of European sea bass, *Dicentrarchus labrax*, L. (1758), reared in cages of different volumes. Aquaculture Research, 48: 990–1005.

Samaras, A., Papandroulakis, N., Lika, K., and Pavlidis, M. 2018a. Water temperature modifies the acute stress response of European sea bass, *Dicentrarchus labrax* L. (1758). Journal of Thermal Biology, 78: 84–91.

Samaras, A., Espírito Santo, C., Papandroulakis, N., Mitrizakis, N., Pavlidis, M., Höglund, E., Pelgrim, T. N. M., *et al*. 2018b. Allostatic Load and Stress Physiology in European Seabass (*Dicentrarchus labrax* L.) and Gilthead Seabream (*Sparus aurata* L.). Frontiers in Endocrinology, 9.

Sammouth, S., D'Orbcastel, E. R., Gasset, E., Lemarié, G., Breuil, G., Marino, G., Coeurdacier, J. L., *et al*. 2009. The effect of density on sea bass (*Dicentrarchus labrax*) performance in a tank-based recirculating system. Aquacultural Engineering, 40: 72–78.

Sánchez-Muros, M. J., Sánchez, B., Barroso, F. G., Toniolo, M., Trenzado, C. E., and Sanz Rus, A. 2017. Effects of rearing conditions on behavioural responses, social kinetics and physiological parameters in gilthead sea bream *Sparus aurata*. Applied Animal Behaviour Science, 197: 120–128.

Sánchez-Vázquez, F. J., Azzaydi, M., Martínez, F. J., Zamora, S., and Madrid, J. A. 1998. Annual rhythms of demand-feeding activity in sea bass: Evidence of a seasonal phase inversion of the diel feeding pattern. Chronobiology International, 15: 607–622.

Sánchez, J. A., López-Olmeda, J. F., Blanco-Vives, B., and Sánchez-Vázquez, F. J. 2009. Effects of feeding schedule on locomotor activity rhythms and stress response in sea bream. Physiology and Behavior, 98: 125–129.

Sangiao-Alvarellos, S., Guzmán, J. M., Láiz-Carrión, R., Míguez, J. M., Martín Del Río, M. P., Mancera, J. M., and Soengas, J. L. 2005. Interactive effects of high stocking density and food deprivation on carbohydrate metabolism in several tissues of gilthead sea bream *Sparus auratus*. Journal of Experimental Zoology Part A: Comparative Experimental Biology, 303A: 761–775.

Santos, G. A., Schrama, J. W., Mamauag, R. E. P., Rombout, J. H. W. M., and Verreth, J. A. J. 2010. Chronic stress impairs performance, energy metabolism and welfare indicators in European seabass (*Dicentrarchus labrax*): The combined effects of fish crowding and water quality deterioration. Aquaculture, 299: 73–80.

Sarà, G., Oliveri, A., Martino, G., and Campobello, D. 2010. Changes in behavioural response of Mediterranean seabass (*Dicentrarchus labrax* L.) under different feeding distributions. Italian Journal of Animal Science, 9: e23.

Sfakianakis, D. G., Georgakopoulou, E., Papadakis, I. E., Divanach, P., Kentouri, M., and Koumoundouros, G. 2006. Environmental determinants of haemal lordosis in European sea bass, *Dicentrarchus labrax* (Linnaeus, 1758). Aquaculture, 254: 54–64.

Sfakianakis, D. G., Katharios, P., Tsirigotakis, N., Doxa, C. K., and Kentouri, M. 2013. Lateral line deformities in wild and farmed sea bass (*Dicentrarchus labrax*, L.) and sea bream (*Sparus aurata*, L.). Journal of Applied Ichthyology, 29: 1015–1021.

Shrivastava, J., Ndugwa, M., Caneos, W., and De Boeck, G. 2019. Physiological trade-offs, acid-base balance and ion-osmoregulatory plasticity in European sea bass (*Dicentrarchus labrax*) juveniles under complex scenarios of salinity variation, ocean acidification and high ammonia challenge. Aquatic Toxicology, 212: 54–69.

Singer, P. 1975. Animal Liberation: A New Ethics for Our Treatment of Animals. Harper Collins, New York.

Sinha, A. K., Dasan, A. F., Rasoloniriana, R., Pipralia, N., Blust, R., and De Boeck, G. 2015. Hypo-osmotic stress-induced physiological and ion-osmoregulatory responses in European sea bass (*Dicentrarchus labrax*) are modulated differentially by nutritional status. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 181: 87–99.

Sitjà-Bobadilla, A., and Pérez-Sánchez, J. 1999. Diet related changes in non-specific immune response of European sea bass (*Dicentrarchus labrax* L.). Fish and Shellfish Immunology, 9: 637–640.

Skalli, A., Robin, J. H., Le Bayon, N., Le Delliou, H., and Person-Le Ruyet, J. 2006. Impact of essential fatty acid deficiency and temperature on tissues' fatty acid composition of European sea bass (*Dicentrarchus labrax*). Aquaculture, 255: 223–232.

Skrzynska, A. K., Gozdowska, M., Kulczykowska, E., Martínez-Rodríguez, G., Mancera, J. M., and Martos-Sitcha, J. A. 2017. The effect of starvation and re-feeding on vasotocinergic and isotocinergic pathways in immature gilthead sea bream (*Sparus aurata*). Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology, 187: 945–958.

Sneddon, L. U., Braithwaite, V. A., and Gentle, M. J. 2003. Do fishes have nociceptors? Evidence for the evolution of a vertebrate sensory system. Proceedings of the Royal Society of London. Series B: Biological Sciences, 270: 1115–1121.

Sneddon, L. U. 2019. Evolution of nociception and pain: evidence from fish models. Philosophical Transactions of the Royal Society B: Biological Sciences, 374: 20190290.

Soares, S., Green, D. M., Turnbull, J. F., Crumlish, M., and Murray, A. G. 2011. A baseline method for benchmarking mortality losses in Atlantic salmon (*Salmo salar*) production. Aquaculture, 314: 7–12.

Sola, L., Innocentis, S. De, Rossi, A. R., Crosetti, D., and Scarcl, M. 1998. Genetic variability and fingerling quality in wild and reared stocks of European sea bass, *Dicentrarchus labrax*. CIHEAM - Options Mediterraneennes, 280: 273–280.

Somarakis, S., Pavlidis, M., Saapoglou, C., Tsigenopoulos, C. S., and Dempster, T. 2013. Evidence for 'escape through spawning' in large gilthead sea bream *Sparus aurata* reared in commercial sea-cages. Aquaculture Environment Interactions, 3: 135–152.

Spiga, I., Aldred, N., and Caldwell, G. S. 2017. Anthropogenic noise compromises the anti-predator behaviour of the European seabass, *Dicentrarchus labrax* (L.). Marine Pollution Bulletin, 122: 297–305.

Stewart, A., Gaikwad, S., Kyzar, E., Green, J., Roth, A., and Kalueff, A. V. 2012. Modeling anxiety using adult zebrafish: A conceptual review. Neuropharmacology, 62: 135–143.

Szisch, V., Papandroulakis, N., Fanouraki, E., and Pavlidis, M. 2005. Ontogeny of the thyroid hormones and cortisol in the gilthead sea bream, *Sparus aurata*. General and Comparative Endocrinology, 142: 186–192.

Tandler, A., Anav, F. A., and Choshniak, I. 1995. The effect of salinity on growth rate, survival and swimbladder inflation in gilthead seabream, *Sparus aurata*, larvae. Aquaculture, 135: 343–353.

Thetmeyer, H., Waller, U., Black, K. D., Inselmann, S., and Rosenthal, H. 1999. Growth of european sea bass (*Dicentrarchus labrax* L.) under hypoxic and oscillating oxygen conditions. Aquaculture, 174: 355–367.

Thibaut, L. H., Emilie, F., Béatrice, C., Romain, G., François, R., Sophie, H., Eva, B. B., *et al*. 2019. Are European sea bass as euryhaline as expected? Intraspecific variation in freshwater tolerance. Marine Biology, 166: 1–16.

Tort, L., Sunyer, J. O., Gómez, E., and Molinero, A. 1996. Crowding stress induces changes in serum haemolytic and agglutinating activity in the gilthead sea bream *Sparus aurata*. Veterinary Immunology and Immunopathology, 51: 179–188.

Tort, L., Pavlidis, M. A., and Woo, N. Y. S. 2011. Stress and Welfare in Sparid Fishes. In Sparidae: biology and aquaculture of gilthead sea bream and other species, pp. 75–94. Ed. by M. Pavlidis and C. C. Mylonas. Wiley-Blackwell, Oxford, UK.

Tort, L., Rotllant, J., Pavlidis, M., and Montero, D. 2014. The response to stressors in the sea bass. In Biology of European Sea Bass, pp. 374–400. Ed. by F. J. S. Vazquez and J. A. Munoz-Cueto. CRC Press, Boca Raton.

Trocino, A., Xiccato, G., Carraro, L., Simontacchi, C., and Poltronieri, C. 2005. Effect of pre-slaughter conditions in European sea bass (*Dicentrarchus labrax*). Italian Journal of Animal Science, 4: 606–608.

Tsalafouta, A., Papandroulakis, N., and Pavlidis, M. 2015a. Early life stress and effects at subsequent stages of development in European sea bass (*D. labrax*). Aquaculture, 436: 27–33.

Tsalafouta, A., Papandroulakis, N., Gorissen, M., Katharios, P., Flik, G., and Pavlidis, M. 2015b. Ontogenesis of the HPI axis and molecular regulation of the cortisol stress response during early development in *Dicentrarchus labrax*. Scientific Reports, 4: 5525.

Tsalafouta, A., Sarropoulou, E., Papandroulakis, N., and Pavlidis, M. 2018. Characterization and expression dynamics of key genes involved in the gilthead sea bream (*Sparus aurata*) cortisol stress response during early ontogeny. Marine Biotechnology, 20: 611–622.

Vail, A. L., Manica, A., and Bshary, R. 2014. Fish choose appropriately when and with whom to collaborate. Current Biology, 24: R791–R793.

van de Vis, H., Bergevoet, R., Stokkers, R., Schrijver, R., Dewar, D., van de Braak, K., and Witkamp, S. 2017. Welfare of farmed fish: Common practices during transport and at slaughter. 186 pp.

van de Vis, H., Kestin, S., Robb, D., Oehlenschläger, J., Lambooij, B., Münkner, W., Kuhlmann, H., *et al*. 2003. Is humane slaughter of fish possible for industry? Aquaculture Research, 34: 211–220.

Vanderplancke, G., Claireaux, G., Quazuguel, P., Huelvan, C., Corporeau, C., Mazurais, D., and Zambonino-Infante, J.-L. 2015. Exposure to chronic moderate hypoxia impacts physiological and developmental traits of European sea bass (*Dicentrarchus labrax*) larvae. Fish Physiology and Biochemistry, 41: 233–242.

Vapnek, J., and Chapman, M. 2011. Legislative and regulatory options for animal welfare. Rome. 34 pp.

Vardar, H., and Yildirim, S. 2012. Effects of long-term extended photoperiod on somatic growth and husbandry parameters on cultured gilthead seabream (*Sparus aurata*, L.) in the net cages. Turkish Journal of Fisheries and Aquatic Sciences, 12: 5.

Velázquez, M., Zamora, S., and Martínez, F. J. 2004. Influence of environmental conditions on demand-feeding behaviour of gilthead seabream (*Sparus aurata*). Journal of Applied Ichthyology, 20: 536–541.

Vendramin, N., Zrncic, S., Padrós, F., Oraic, D., Le Breton, A., Zarza, C., and Olesen, N. J. 2016. Fish health in Mediterranean Aquaculture, past mistakes and future challenges. Bulletin of the European Association of Fish Pathologists, 36: 38–45.

Vera, L. M., Davie, A., Taylor, J. F., and Migaud, H. 2010. Differential light intensity and spectral sensitivities of Atlantic salmon, European sea bass and Atlantic cod pineal glands ex vivo. General and Comparative Endocrinology, 165: 25–33.

Vera, L. M., Montoya, A., Pujante, I. M., Pérez-Sánchez, J., Calduch-Giner, J. A., Mancera, J. M., Moliner, J., *et al*. 2014. Acute stress response in gilthead sea bream (*Sparus aurata* L.) is time-of-day dependent: Physiological and oxidative stress indicators. Chronobiology International, 31: 1051–1061.

Villamizar, N., Blanco-Vives, B., Migaud, H., Davie, A., Carboni, S., and Sánchez-Vázquez, F. J. 2011. Effects of light during early larval development of some aquacultured teleosts: A review. Aquaculture, 315: 86–94.

Wendelaar Bonga, S. E. 1997. The stress response in fish. Physiological Reviews, 77: 591–625.

Yan, H., Liu, Q., Cui, X., Shen, X., Hu, P., Liu, W., Ge, Y., *et al*. 2019. Growth, development and survival of European sea bass (*Dicentrarchus labrax*) larvae cultured under different light spectra and intensities. Aquaculture Research, 50: 2066–2080.

Yildirim, Ş., and Vardar, H. 2015. The influence of a longer photoperiod on growth parameters of European sea bass *Dicentrarchus labrax* (Linnaeus, 1758) reared in sea cages. Journal of Applied Ichthyology, 31: 100–105.

Yilmaz, H. A., Turkmen, S., Kumlu, M., Eroldogan, O. T., and Perker, N. 2020. Alteration of growth and temperature tolerance of european sea bass (*Dicentrarchus labrax* linnaeus 1758) in different temperature and salinity combinations. Turkish Journal of Fisheries and Aquatic Sciences, 20: 331–340.

Zambonino-Infante, J. L., Mazurais, D., Dubuc, A., Quéau, P., Vanderplancke, G., Servili, A., Cahu, C., *et al.* 2017. An early life hypoxia event has a long-term impact on protein digestion and growth in juvenile European sea bass. The Journal of Experimental Biology, 220: 1846–1851.

Zampacavallo, G., Scappini, F., Mecatti, M., Iurzan, F., Mosconi, G., and Poll, B. M. 2003. Study on methods to decrease the stress at slaughter in farmed sea bass (*Dicentrarchus labrax*). Italian Journal of Animal Science, 2: 616–618.

Zanuy, S., Carrillo, M., and Ruiz, F. 1986. Delayed gametogenesis and spawning of sea bass (*Dicentrarchus labrax* L.) kept under different photoperiod and temperature regimes. Fish Physiology and Biochemistry, 2: 53–63.

Zohar, Y., Abraham, M., and Gordin, H. 1978. The gonadal cycle of the captivity-reared hermaphroditic teleost *Sparus aurata* (L.) during the first two years of life. Ann. Biol. Anim. Bioch. Biophys., 18: 877–882.

Zohar, Y., and Gordin, H. 1979. Spawning kinetics in the gilthead sea-bream. Journal of Fish Biology: 665–670.

[Bibliography in Greek]

Athanasopoulou, Ph. 2001. Τα κυριότερα παρασιτικά νοσήματα των εκτρεφόμενων ελληνικών θαλάσσιων ψαριών. Δελτίον Ελληνικής Κτηνιατρικής Εταιρείας [= The main parasitic diseases observed in marine cultured fish in Greece. Journal of the Hellenic Veterinary Medical Society], 52: 9–17.

FGM 2018. ΣΕΘ Ελληνική Υδατοκαλλιέργεια Ετήσια Έκθεση [= Federation of Greek Maricultures. Greek Mariculture Annual Report].

Haziroglou, L. 2018. Δικαιώματα των ζώων και ηθική: Θεωρίες και πράξη. Εκδόσεις Άπαρσις. [= Animal rights and ethics: Theories and practice. Aparsis Press]. 128 pp.